

nature*November 13, 1975*

Asking questions about university research . . .

UNIVERSITY bashing is a popular pastime in the UK at the moment. True, all forms of public expenditure are bound to come under scrutiny when a country is trying to keep control of a serious economic situation, but the universities are rather better than average sitting targets because it is much easier to question the expenditure of several hundred million pounds on them than to justify it in a way that convinces the holders of the purse strings. On a slightly more detailed level, asking a question like: "Why do that particular kind of scientific research?" is easy; answering it is not.

Nobody pretends, of course, that one can find out much about as complex a beast as 'university research' by talking to two, or even ten, people. The Science Subcommittee of the House of Commons Select Committee on Science and Technology, for example, must be truly fed up with asking a question of a witness, only to be told that the query should be addressed to someone else (usually unspecified) because it is outside that witness's brief, remit or responsibility. Equally, 'ologists' of the Select Committee on Science and Technology often feel that key questions are not asked or pursued—often, it must be added in the select committee's defence, because time simply does not permit.

The objections of many of those who carp about the amounts spent on scientific research in universities stem from the supposed antipathy between universities and industry. The stereotyped picture is one in which industry

complains that university training in research often does not suit the industrial environment; the universities reply that a major part of their function is to acquire new knowledge and that industry should not expect a model industrial scientist to step off a university production line.

Fortunately things are not nearly as polarised as that, but there certainly are problems, and it is therefore a particularly important event when the select committee talks to industry, be it the Confederation of British Industry (CBI) or individual industrialists, about university research. It so happens that the select committee had the CBI before it last week, and one is therefore prompted to set up a list of some of the more important matters that might be covered in future select committee discussions with industry. Some of these came up at the recent meeting; some did not.

- How can the link between 'research' and 'application' be made less tenuous?
- How can the requirements of industry for, broadly, 'trained' people be squared with the universities' aim of producing 'educated' people?
- To what extent is university research compatible with the goal of much of industrial effort ('improvement' as opposed to 'innovation')?
- In what light do industrialists regard polytechnics? Should they be encouraged at the expense of (some) universities?

. . . at Cambridge in particular

In the concluding remarks of a paper on 'useful' research carried out at the University of Cambridge (distributed from the University Offices) the late Professor J. W. Linnett said: "I have been surprised at the amount and range of useful research that is being done at Cambridge over a very wide spread of disciplines", when 'usefulness' is judged "in economic and social terms of a simple and direct kind".

This is an important statement for a man who was until a month or so ago Vice-Chancellor of the University

of Cambridge to have made, even allowing that he had set out to tackle the problem of bolstering up the public image of universities. Interestingly, the standard university response to critics is also contained in Professor Linnett's paper: "Universities are places where the spirit of unfettered imaginative enquiry should be fostered". Adding his two statements together leads to a very important conclusion—that unfettered imaginative enquiry in a university environment can be of considerable use in a tangible way.





Preparing polymerisers for cleaning at BP factory.

Cutting out cancers

THE UK Government recently accepted a Convention and a Recommendation, drawn up at last year's International Labour Conference, on 'international standards concerning the protection of workers against carcinogenic substances or agents'. Both documents will play their part in establishing some useful principles and eliminating past abuses. But the practical achievement will fall short of what is usually understood by 'international standards', and the proportion of actual cancers prevented is likely to be only a fraction of the potential number. Laura Swaffield reports.

THE terms set out by the International Labour Organisation (ILO) must be seen in context. First, the documents can apply only to substances known to be carcinogenic. Unfortunately, the probability is that most industrial carcinogens have not been identified as such.

The International Agency for Research on Cancer takes it as a rule of thumb that 80% of all cancers are environmentally induced, the most easily traced often by chemicals at work. According to the WHO, however, adequately documented cases of occupational cancer represent only a fraction of 1% of the total.

The medical profession's notorious preoccupation with finding a dramatic breakthrough cure is partly to blame. A sub-committee of the US National Cancer Advisory Board, which expressed its astonishment in March of this year that "the National Cancer Pro-

gram does not appear to have accorded an adequate priority nor sense of urgency to the field of environmental carcinogenesis", estimated research expenditure on preventive work as "perhaps 10% of the budget"—a pattern which is fairly typical worldwide, and not just in cancer research.

Second, the ILO documents allow great latitude to the nations concerned—each, for instance, decides for itself which carcinogens are to be prohibited or controlled, and the nature of the controls.

Third, sheer economic reality places limits on how much can be done in industry. This is one reason why individual nations are given so much freedom to decide priorities.

The whole matter came under discussion at this year's XVIIIth International Congress on Occupational Health, held in Brighton. Dr E. Mastromatteo of the ILO said the

future need was for international agreements on exposure levels to chemicals. But Mr M. El Batawi from WHO was more aware of financial, political and economic problems, especially in underdeveloped countries. International exposure levels, he said, were neither practicable nor desirable—but standardised international methodology could perhaps establish a 'dose-response curve' along which each government could set its own limits.

Two case histories from the UK illustrate the bounds between which a line must be drawn. In 1927, ICI patented Nonox S, an anti-oxidant containing 2-naphthylamine which was marketed to the rubber industry. By 1938, the Association of British Chemical Manufacturers had accepted that there was a cancer risk from 2-naphthylamine. ICI started screening its own Nonox S workers, and by 1942 had established a proved cancer hazard for those working with the hot finished product. Only in 1949 was Nonox S withdrawn from sale.

The story does not end there. There was no warning to customers, and one, Dunlop, waited until the mid-1950s to set up a research unit, and until 1960 to recommend the screening of employees who had worked with Nonox S. At one plant, screening did not begin until 1965. By 1970, some 450 cases of bladder cancer had come to light many too late for effective treatment.

As late as this year, the National Health Service (NHS) distributed a circular setting out the screening arrangements for workers and ex-workers in the rubber industries. Some workers who left their jobs before screening started must still be unaware they are at risk. Meanwhile, oddly enough, the NHS does not want the circular to attract great publicity because it has "limited application", even though its implications are vast and of great discredit to all concerned.

The second case, the big occupational cancer story of 1974-75, involves vinyl chloride monomer (VCM) and shows the other side of the coin. Employees' organisations were consulted from the start when a link was discovered between VCM (used in the manufacture of PVC) and angiosarcoma, a rare liver cancer. The discovery, typically, came by accident during animal research on a VCM-linked hand condition. By early 1974, epidemiological work had turned up a possible four VCM-linked angiosarcomas in the USA, and one in the UK.

The ensuing events are something of a demonstration of ideal industrial practice. The chemical industry and government agencies (in the UK, the Employment Medical Advisory Service of the new Health and Safety Com-

mission) got together, with trade union involvement, to find out the extent of the danger and eliminate it. The then medical adviser to the Trades Union Congress (TUC), pointed out from the start that there was no case for shutting down the polymerisation process, which annually produces 10 million tons of PVC world-wide.

By June 1974, the Department of Employment was drawing up a code of practice limiting exposure to VCM vapour to 50 parts per million with a time-weighted average of 25 ppm. As late as 1959, the prescribed threshold limit value had been 500 ppm. Significantly, the code of practice required results of atmospheric sampling to be available to workers. Today, the limit in industry is fixed at 30 ppm. (time-weighted average 10 ppm.), which is thought to be the ultimate with existing plant.

This shows what industry can do if it tries. But how does the balance sheet look? In the UK alone, £13 million has been spent since 1974 on research and alterations to plant. Four companies with a total of 500 workers are involved.

But the total of deaths in the UK now found to have been due to VCM-induced angiosarcoma is two—one a man of 71, the other a man with a long history of liver abnormality. The world-wide score is under 40, not all of them confirmed.

With thousands of new chemicals coming into use every year, it is only too clear that any attempt to trace and control all carcinogens, even where deaths have already occurred, would bankrupt the world chemical industry in no time. It is up to individual nations, therefore, to decide what is an acceptable risk, socially and economically.

The ILO Convention, within these limits, requires "every effort" to be made to replace or control carcinogens, all available information to be given to exposed or formerly exposed workers, relevant tests to be carried out on all workers (although there is no provision that the results be communicated to them) and government supervision throughout.

There is also an important requirement that "an appropriate system of records" be kept. Death notifications and employers' records have been instrumental in tracing VCM and Nonox S workers, and record systems are the essence of occupational control where a carcinogen is discovered.

The Recommendation—a less binding formulation for signatories than the Convention—goes much further than the Convention. Most of the extras concern information: the national "competent authority" is to promote

HEALTH and safety legislation in the UK has traditionally been of the Old Testament type—detailed rules with penalties for non-compliance. This had its disadvantages. The Factory Inspectorate, for example, being hopelessly undermanned, was forced into a soft-footed approach rather than waste valuable inspecting time in court taking prosecutions which led to derisory fines even for fatal negligence.

Progress came through the Common Law which enshrines a New Testament-style duty to care for one's neighbour and leaves the practical details to the individual. It was under Common Law, for instance, that two Dunlop employees with bladder cancer were able to obtain damages in 1971 from ICI, which had no legal responsibility to them under the Factories Acts.

Claims for damages have done much to clarify the responsibilities of both employers and employees. Moreover, it was the first successful damages claim for hearing loss induced by noise at work which led to a business boom for the manufacturers of protective equipment—and has made the Department of Employment's code of practice on noise (not statutorily binding) a best-seller.

Purpose-built Old Testament legislation is usually narrow in its usefulness, and in modern industrial conditions may be out of date as soon as the ink is dry. In the UK the new Health and Safety at Work Act, which came into force this year, and unifies the Factory and Alkali Inspectorates into one Safety and Health Executive, goes as far towards the flexible New Testament approach as is possible.

It imposes a slightly more detailed duty of care on employer, employee and supplier. But old detailed regulations built up over a century remain in force, and will be expanded,

together with the more recently produced codes of practice.

Replacing the old penalties is a system of improvement and prohibition notices which oblige a manufacturer actually to shut down a process until it is made safe to the satisfaction of the relevant inspector. Arguments about notices are dealt with by the industrial tribunals, leaving the inspector to inspect and hand out notices. This puts a high price on failure to comply with the inspector's recommendations. In serious cases, the executive is free to prosecute on indictment, and the duty of care makes it reasonably clear who is to be prosecuted in each case taken. A jury verdict of guilty will lead to an unlimited fine, or perhaps even to imprisonment.

The picture elsewhere in the world mirrors the unsatisfactory state of pre-1975 UK legislation. Most EEC countries have detailed regulations about the provision of occupational health services in industrial companies. In practice, however, the carefully provided nursing services do little except routine curative work, which leaves out preventive work and epidemiology.

The USA has far-reaching and stringent safety and health laws on the Old Testament line—plus a tiny inspectorate which, it is estimated, is able to visit each factory about once every 400 years.

In the USSR, where there is no clear distinction between employer and employee in industry, the unions lack the sort of power exercised in the UK and the USA. Overall responsibility for safety and health is with government and the unions. Numerous state agencies are also involved—44 of them, for instance, in the development of the Lada (Fiat) car factory under licence from Italy. The result is often confusion and, occasionally, inaction.

epidemiological studies, collect and disseminate all available information, produce "educational guides" for both employers and employees, and try to establish criteria for determining carcinogenicity.

Employers have a duty to seek out information, carry out studies with the aid of the competent authority, notify all affected workers and instruct them regularly. Employers' and workers' organisations both have a duty to carry out information programmes and encourage full participation by workers in control programmes.

The Nonox S story shows how necessary such measures can be, especially in countries (like the UK in the 1940s) with an undeveloped system of safety

law enforcement and worker participation.

But implementation could also be a tall order in many countries, and even the UK, which now probably leads the world in health and safety legislation, has confessed that 'it will take a considerable time to set up suitable systems.'

International cooperation on occupational health research is acknowledged to be very poor at present, quite apart from the unsatisfactory distribution of the research effort itself. The ILO's ideas on information could point the way to making use of what information there is. But it seems a pity they are tucked away in a mere Recommendation. □

Nobel award to Prelog

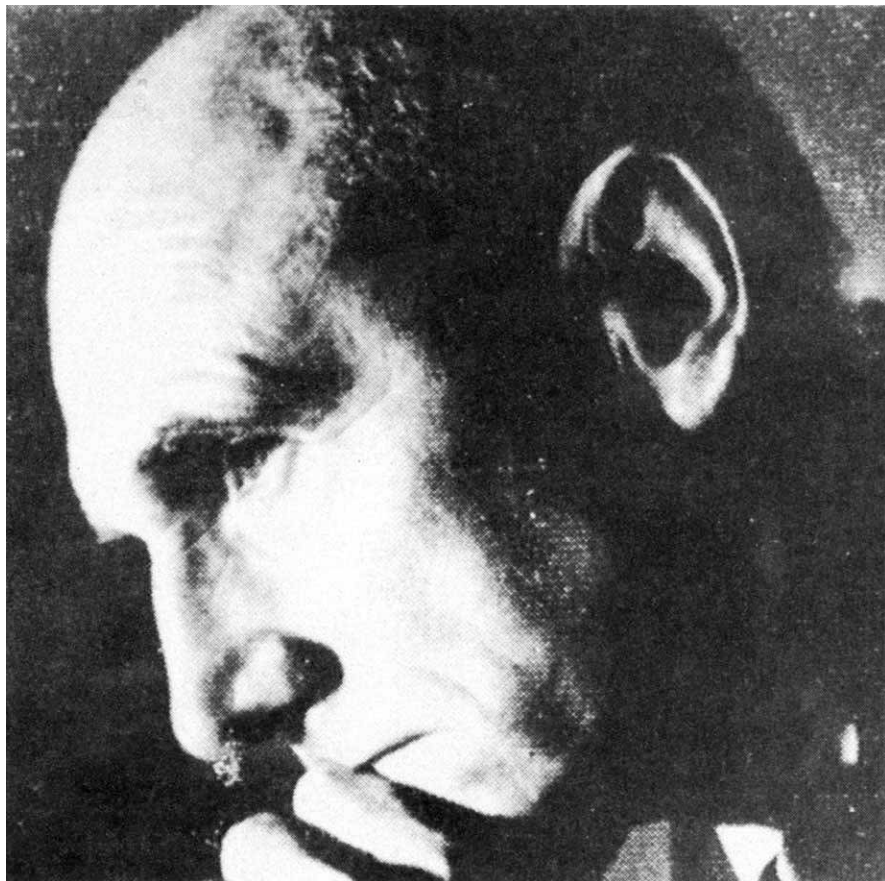
PROFESSOR Vladimir Prelog of the Federal Technical High School (ETH) in Zürich has been awarded half of the Nobel Prize for Chemistry, at the age of 69, for his work on stereochemistry. It comes to him not so much for any one outstanding discovery, but for a lifetime's work in chemistry which has produced a steady succession of significant researches combining deep thought and elegant experimental work. van't Hoff is considered as the founder of 'Chemistry in space'; Prelog has been the High Priest of this cult for the past 20 years.

Prelog was born in 1906 in Sarajevo in the Austro-Hungarian Empire. He studied in Prague, worked in industry, and then joined the staff of the University of Zagreb in Yugoslavia. Fortunately for Prelog and for chemistry, he found his way to Switzerland in 1941 and joined the great ETH School of Organic Chemistry. His career in Zürich was naturally influenced by his predecessor, Professor L. Ruzicka (Nobel Laureate, 1939), whom he succeeded in 1957. Prelog's structural and synthetic studies on natural products have covered a wide range, including terpenes, steroids, alkaloids and antibiotics.

A major stereochemical interest started from the preparative work of the Zürich school on medium and large rings, and developed into Prelog's studies on transannular reactions. In the spirit of his time, Prelog not only made new compounds by ingenious methods, but studied their physical properties and their reactivity, using all of the steadily increasing range of tools provided by physical chemistry.

His work in stereochemistry has included a considerable share in the study of conformations, for example, his pioneer work on asymmetric synthesis and the medium-ring work noted here. He delivered one of the earliest Centenary Lectures to the Chemical Society in London in 1949; this significantly influenced the thinking of other pioneers of conformational studies.

Prelog's ability to get to the heart of things is nowhere better illustrated than in the work on the Sequence Rule. This work, the result of a very fruitful partnership between Prelog, the late Sir Christopher Ingold and Dr R. S. Cahn, soon developed into something much more than a method for designating stereoisomers (the "*R* and *S*" system). It led Prelog to consider in a simple, but fundamental way, the



Vladimir Prelog

geometrical basis of stereochemistry.

The Sequence Rule papers first showed clearly and in a systematic framework of thought the nature of axes and planes of chirality. (The term "chirality", borrowed from Kelvin, has been universally accepted as a convenient term for the property of "handedness".) The need to test the Sequence Rule exhaustively during its development caused Prelog to visualise, and then to make with his students, organic compounds of widely differing types displaying stereoisomerism of kinds apparently unknown in nature, and hitherto unsuspected by organic chemists—for example, cycloenantiomers and cyclodiastereoisomers.

In all of this, Prelog has carried to its logical conclusion the concept of the tetrahedral atom (van't Hoff and Le Bel). The marriage of chemistry and geometry thus arranged by Prelog, and called by him "chemical topology" is already a happy and most fruitful one. All types of stereoisomerism can be derived from the consideration of simplexes in two- and three-dimensional space, alone or in combination.

The general principles were outlined in lectures in Amsterdam, in Dublin, in Hamburg, and in Zürich (Karrer Lecture), and were presented in a more extended form in lectures at Cornell. Some of these ideas have now appeared in a very general paper modestly entitled "Pseudoasymmetry in organic chemistry", which was followed by

two detailed papers on compounds showing axes and planes of pseudoasymmetry, giving some experimental 'meat' to cover the elegant geometrical skeleton. The international group characteristically addressed by Prelog in letters as *Stereofreunde* await with great interest and some impatience the fuller story.

Much of Prelog's recent work has been on the borders of chemistry and biochemistry, including his studies on enzymatic reactions. In addition to their supreme biochemical importance, enzymatic reactions provide a suitable terrain for thinking in terms of two-dimensional chirality.

Like many continental professors, Prelog has long had close connections with industry, and he has been for some years a Director of Ciba-Geigy in Basel.

He is a frequent and welcome visitor to laboratories all over the world—and nowhere more welcome than in the UK. Few chemists offer more stimulating discussion to colleagues of all ages and interests. He is always one of the liveliest disputants at any meeting, and he was one of the founding fathers of the annual Bärnstock Conferences on Stereochemistry.

The *Stereofreunde* rejoice in this award to Prelog. Perhaps the pilgrimage to Stockholm will usher in an age of many-dimensional stereochemical thinking.

W. Klyne

international news

IT was just two years ago that former President Nixon coined the term Project Independence as the rallying cry for the drive to make the United States self-sufficient in energy. Since then funds for energy research and development have boomed, the Energy Research and Development Administration has been established to take charge of the effort, and President Ford has reiterated at every opportunity his commitment to the goal of reducing oil imports. Although Mr Nixon's optimistic predictions that the nation could achieve energy independence by 1980 were quickly written off as unrealistic political hyperbole, self-sufficiency is still the guiding principle behind energy policy in the United States.

But, in a report which pulls few punches and which probably represents the most thorough independent analysis so far performed on the federal government's plans for energy research and development, Congress's new Office of Technology Assessment (OTA) has warned that ERDA's strategy is "inadequate" and that it has defects which could even "lead to an increased dependence on foreign energy sources". OTA has raised two broad criticisms of ERDA's programmes, and it has also faulted the agency on a host of specific points.

Prepared by six panels of experts drawn from universities, industry and non-profit institutions, the OTA study concentrated its attention on a massive two-volume plan and programme published by ERDA last June, which will form the basis of the federal government's energy research and development operations over the next few years (see *Nature*, 256, 83; 1975). It should be noted in ERDA's defence, however, that the plan was published less than six months after ERDA was established, and it was prepared while the agency was recruiting staff and finding its feet. Inevitably, the detailed strategy reflects the sum total of the efforts which ERDA inherited from other agencies, and it's not surprising that the programmes show few significant changes in direction. Indeed, it was no small feat that ERDA managed to produce the plan at all in such a short period.

Nevertheless, OTA's two chief criticisms go to the heart of ERDA's strategy, and they merit close attention. To begin with, OTA said that

US energy policy criticised

by Colin Norman, Washington

ERDA's overall plan "is a significant milestone in the evolution of a long-term national energy policy", but it found that the programmes which ERDA is supporting do not "appear adequate to achieve the stated goals". The problem, OTA suggests, is that ERDA has taken too narrow an approach by concentrating almost exclusively on the development of sophisticated technology and neglecting many non-technical factors which could inhibit the implementation of new technologies. Moreover, OTA has accused ERDA of paying insufficient attention to conservation by failing to promote the development of relatively mundane technologies which could reduce energy growth in the short term.

"In addressing the energy goals, ERDA adopted a narrow, hardware-oriented approach," OTA suggests. Although such an approach may be well suited to putting a man on the Moon, or building new weaponry, OTA notes that the energy crisis is "a far more complex and wide ranging challenge". As a result, "ERDA's narrow approach to the national energy policy goals might well fulfill a mission—developing new technology—without providing an answer—a secure energy future. Unresolved 'nontechnical' issues—from inadequate incentives for commercialisation through environmental demands, competitive use of resources, to community resistance—could block the most sophisticated engineering achievement", OTA warns.

ERDA officials are, of course, well aware of such criticisms. When the plan was published last June, for example, Dr Robert C. Seamans Jr., the Administrator of ERDA, stated explicitly that "it is one thing to take a relatively small number of astronauts on an experimental-type mission. It is another thing to put into being in this country an energy delivery system and an energy conservation system which will satisfy the needs of over 200 million people . . . It is a much more complex problem we face than just the research and development".

OTA clearly feels, however, that although ERDA may recognise the need for a broad approach to energy research and development, its plan does not reflect that need. "If ERDA confines its activities predominantly to the proving of the feasibility of technological options, some other entity should address the more complex issues underlying energy solutions," OTA suggests.

A central difficulty, which OTA does not specifically address in its report, however, is that overall energy policy in the United States is hopelessly confused. Congress and the White House have been at loggerheads for months on the question of whether or not oil and gas prices should be decontrolled, for example, and Congress has not yet demonstrated that it has the stomach for taking politically unpopular decisions to curb energy growth in an election year. Energy policy, in short, has become a political football, and swift progress on the "more complex problems underlying energy solutions" should not be anticipated.

As for OTA's more specific complaints about ERDA's plans, the following are among the more important:

- Only about 2% of ERDA's budget is allocated to conservation programmes, yet "successful widespread implementation of conservation programmes . . . can have both a rapid and continuing effect", OTA suggests. ERDA may be neglecting such efforts because that are relatively mundane, and not intellectually as exciting as the development of sophisticated new supply technologies.

- Insufficient emphasis is placed on international cooperation in such areas as nuclear waste disposal, the management of nuclear materials, and the development of ocean resources.

- ERDA is not devoting enough attention to commercialisation of new energy technology. Although ERDA's plan outlines a philosophy for commercialisation, it "clearly needs a more detailed explanation and careful definition of plans for developing a mechanism for coordination with industry".

- "Only limited attention is given to socioeconomic research and analysis in addressing the nation's energy problems". Broad-ranging research is needed to identify non-technological obstacles to energy solutions.

● Conventional wisdom is that electrical energy consumption will continue to grow as a much faster rate than total energy consumption. But OTA notes that "intensive electrification will have a noticeable social impact and may present problems of vulnerability and reliability", and suggests that "the long-term electrification approach should be more thoroughly analysed . . . to make sure that viable alternatives are not lost by default".

OTA's analysis was performed at the request of three Congressional committees which have jurisdiction over ERDA, and which are thus in a sound position to ensure that the agency changes its policies if necessary. The plan will be updated each year and republished in January along with the President's budget request. □

Re-establishment of science office

AFTER months of agitation by prominent spokesmen for the scientific community, the House of Representatives last week approved a bill to reestablish a science policy office in the White House. The Senate is expected to follow suit early next month, and the measure could be on Mr Ford's desk soon after Christmas—almost exactly three years after Mr Nixon decided that he no longer required the services of a full-time science adviser.

The bill swept through the House to the accompaniment of a chorus of laudatory remarks about the benefits which scientists can bring to the heart of national policymaking, and it was eventually approved by the impressive margin of 362 votes to 28. According to Representative Charles Mosher, a senior republican from Ohio who helped draft the measure, the bill also has Mr Ford's wholehearted support.

Scientists are therefore well on their way back to the inner corridors of power, from which they were banished by Mr Nixon in 1973 as the result of a post-election shake up of the White House staff. Mr Nixon dismantled the Office of Science and Technology, which was established by Presidents Eisenhower and Kennedy, and conferred the title of science adviser on the already overburdened shoulders of the Director of the National Science Foundation, Dr H. Guyford Stever.

Although it was fortunate that the science policy apparatus was out of the White House when it was wallowing in the Watergate morass, there have since been numerous calls—including one from the prestigious National Academy of Sciences—for it to be brought back

in. As a result, Mr Ford five months ago sent Congress a legislative proposal for a rather modest office, headed by a single science adviser.

The bill passed by the House last week conforms rather closely to Mr Ford's proposal, although a few significant changes were made along the way. The office, which would be called the Office of Science and Technology Policy (OSTP), would be limited almost exclusively to advice and analysis—it would have no direct control over scientific programs or budgets. The director of OSTP would be the President's chief science adviser, and his brief would extend over most of the federal government's scientific affairs, including military technology, the economy, foreign relations, and so on. The budget for OSTP would be about \$2 million a year.

As for the manner in which the office would carry out its responsibilities, the bill merely states that the director should "develop appropriate working relationships" with the National Security Council and the Domestic Council and "maintain liaison with" all other units in the President's immediate vicinity. In other words, the office would fit into the White House power structure as best it could.

During the Johnson and Nixon Administrations, the old Office of Science and Technology lost influence largely because of growing disaffection between those presidents and the scientific community, chiefly over the Vietnam War. At the finish, Mr Nixon became agitated because he disagreed with some of the advice he was receiving from the Office, particularly from the President's Science Advisory Committee—a top

level independent committee chaired by the science adviser—and he banished the science advisory functions to the bureaucratic hinterlands.

After that episode, dismayed leaders of the scientific community who felt, in the trenchant words of Nixon's last science adviser Dr Edward E. David, that "science had been downgraded in national affairs", urged that a more permanent structure be established. The fact that OSTP will be set up by legislation effectively means that it cannot be dismantled simply on a Presidential whim—Congress would have to pass a bill to dismantle it.

The bill now goes to the Senate, where it is being handled by three separate committees. Hearings are already under way, and it is expected that a final version can be produced for a vote by the full Senate in December. The chief architect of the Senate's version is likely to be Senator Edward Kennedy, whose subcommittee on the National Science Foundation has a large stake in the enterprise.

Regardless of the form in which the bill eventually emerges from Congress, speculation is already rife in Washington about who will get the job of science adviser when it is established. According to sources within Congress and the Administration, the leading prospect at present is Dr Simon Ramo, an electrical engineer who is vice chairman of the board of TRW Inc, a major defence contractor. Ramo, who is widely respected in science policy circles, could not be reached for comment last week.

● For a few days last week, worried officials of the National Aeronautics and Space Administration (NASA) tried to determine the cause of an electrical failure which threatened to abort one of the two attempts to land a Viking spacecraft on the surface of Mars next year. A battery, which will be needed during the landing operation and which will also be required to power some of the experimental apparatus on the spacecraft, failed to charge when an attempt was made on October 31. The spacecraft was then about 17.6 million km from Earth. The fault was eventually traced to the charging apparatus, and on November 5, a backup charger was used. Fortunately for the \$1,000 million Viking programme, it worked, and NASA officials now believe that the batteries will perform their functions according to plan.

The first Viking spacecraft, whose batteries behaved properly when they were charged last month, is now about 24 million km from Earth. It is expected to arrive at Mars about June 19 next year, and a landing attempt will probably be made on July 4—to coincide with the United States' Bicentennial. □



Guyford Stever

THE government of Ontario, Canada's most populous province, has started an interesting experiment in communal technology assessment this autumn. It has invited its people to help it decide directly how to provide for their future electric power needs.

At a time when economic growth is no longer sacrosanct, when consumer groups are growing increasingly critical of the siting of power stations, the placing of power transmission corridors, and the trend towards the adoption of nuclear energy, such a gesture by the province can only be seen as significant.

The method being used to gather the peoples' opinions is the Royal Commission. The Order-in-Council establishing the Royal Commission on Electric Power Planning was approved last July, and public meetings began on October 28. The commission has been instructed to examine the long range power planning concepts of the public corporation (Ontario Hydro) that is charged with providing the power needs of the province, for the period 1983-93 and beyond. It has been told to relate them to provincial planning, to the utilisation of electrical energy and to environmental, energy and socioeconomic factors, and to report, with a list of priorities, on the need for certain Ontario Hydro projects at present under consideration.

The chairman of the commission is a former professor of engineering at the University of London, Dr Arthur Porter. Porter, who now is professor of industrial engineering at the University of Toronto but on leave for a year, has long been interested in the broader implications of science and technology on society. Serving with him are an industrialist (Robert E. E. Costello, vice-president of corporate services, Abitibi Paper Company Limited), a journalist (Madame Solange Plourde-Gagnon, who will represent the consumer viewpoint), a farmer (George A. McCague, who has served on the executive of many farm organisations) and an economist (Dr William W. Stevenson, at present a member of the Ontario Energy Board).

Announcing the formation of the commission, the Provincial Secretary for Resources Development said: "The inquiry may well be the most important in this decade . . . [it] should bring into public focus basic philosophies about much of the kind of life we want for the next century and what price we are prepared to pay for its achievement . . . The public needs to know what demands for electricity will be placed upon Ontario Hydro in the long term, how these needs should be met, and what impact this would

have on Ontario's way of life and its physical environment."

During the autumn, the commission will hold a series of preliminary public meetings in Ontario in 16 cities. From these it expects to learn what issues the public wants included in the main inquiry, how and in what format it should proceed, and how it can increase public participation. An attempt will be made, the commission says, "to avoid either an inhibiting or court room atmosphere . . . The preliminary meetings in particular will be very informal."

Canadian diary

from David Spurgeon, Ottawa

Some of the major issues and questions expected to arise are:

- Power demands have been increasing by about 7% a year. Should this rate be allowed to continue? What are the chances of its increasing or decreasing?
- How can energy be used more efficiently?
- What are the benefits, costs and risks of alternative ways of generating electrical power? What are the risks associated with nuclear generation, and the environmental implications? How long can we afford to burn irreplaceable fossil fuels?
- How can the important issues associated with land use be decided? Social, economic and environmental factors must be evaluated to decide where industrial and population growth will occur. How should this be done?
- How well are existing procedures working to guarantee public participation in land-use decisions?

One must assume that the hearings will not be equally welcomed by all involved. Recently, Ontario Hydro asked the government for permission to raise its rates by more than 25% because of the rising costs of electricity production, and promptly found itself under heavy fire from the public and politicians. Before that, lengthy and expensive hearings were held on the subject of the placing of power transmission line corridors. And Ontario Hydro's announced intention to rely increasingly on nuclear power has drawn opposition from various quarters. All this has made the power corporation's attempts to fulfil its mandate more difficult.

Dr Porter says he fears the biggest difficulty will be to get ordinary people, outside the major interest groups, to express an opinion—and in fact to achieve good attendances at the public

hearings. A former woman cabinet minister, now heading an inquiry into violence on television, found that only nine people attended a recent public meeting—in spite of what Dr Porter calls a "fantastic press". "If she can't do it," he asks, "how can we?"

The government's move to establish a commission was prompted, says Porter, by a report from Ontario Hydro on their alternative plans for provision of the province's power needs from now until the end of the century. Even assuming the lowest growth rate of power demand considered, the cost came to \$20,000 million; if a 20% growth rate were assumed, on the other hand, the cost amounted to \$50,000 million. With amounts of money like that involved, the corporation felt it had to draw the public into the planning.

● Another Canadian scientist who is trying to get the public involved in decisions involving science and technology is David Suzuki, a geneticist from the University of British Columbia. Five years ago, he recognised the tremendous potential influence of television, and wrote to the Canadian Broadcasting Corporation (CBC) to suggest that it should carry a television series dealing with science and society.

The result was a locally broadcast programme, Suzuki on Science, and then, last year, a job as host of a new programme called Science Magazine. The show was highly successful, but Suzuki says that because there is no basic constituency for science shows, the CBC planned to withdraw it.

According to Suzuki, the viewers objected: several hundred letters of protest were sent to the CBC, and the show will now return in February 1976 for its second season. Suzuki has also begun this year a weekly series of programmes on CBC radio, called Quirks and Quarks. In fact, he has become so busy broadcasting that he has had to take a year's sabbatical leave.

But Suzuki speaks bitterly about his scientific colleagues' interest in reaching the general public. He is cynical about their motives when they do show an interest in reaching the public, and relates it to their need for research funds for their own projects.

"Basically scientists are public servants", he said in a recent interview about another television programme he hosted, called Earthwatch. "I feel our responsibility is to de-mystify the process [of science] so citizens can participate in setting priorities." He does not portray science as being all good, and attempts to show that the scientist as an individual is a human being with all the feelings of his fellow men. □

Soviet euphoria in wake of Venus probes

from Vera Rich

THE success of the two Venus probes, Venera 9 and 10, the orbital sections of which are still gathering and transmitting data, has led to a spirit of self-congratulation, not only among those concerned with the Venus mission, but throughout the Soviet space programme generally.

This success, coming as it does in the concluding months of the present five-year plan, and in a year already distinguished by the Salyut 4 and Soyuz-Apollo missions, will undoubtedly be a considerable incentive towards increasing the space budget in the next plan.

The economic justification for the Soviet space programme, as laid down by the Twenty-fourth Party Congress in 1971, is "to develop long range telephonic-telegraphic communications, television meteorological forecasting and the study of natural resources, geographical investigations and the solutions of other problems of the national economy with the aid of satellites, automatic and piloted spacecraft, and also to carry out fundamental scientific investigations of the Moon and planets of the solar system".

Although basic deep-space research does figure in this list, it seems that a need is felt to justify its importance (and cost) within the framework of the five-year plan. Accordingly, *Pravda* has highlighted not only the political significance of the Venus mission, which illustrates "how rapidly science and technology advances in conditions of socialism", but also the "terrestrial" aspect of the research carried out by the probes—by leading to a better understanding of planetary evolution, they will, it is suggested, facilitate the fuller utilisation of the natural resources of the Earth.

Pravda also carried an article from Professor A. Okhotin, of the Institute of Space Research, on the possibilities of carrying out technological processes in space. This idea has long been of interest to the Soviet space planners—preliminary electron-beam welding and cutting experiments were carried out as long ago as Soyuz 6 in October 1969—and two types of "space technology" are under consideration at present.

The first is the construction of large scale orbital space stations, for astronomical and geodetic observations, prospecting, weather forecasting and environmental surveillance. Tentative plans speak of stations with a mass of hundreds of tonnes and crews of several dozen. Such stations could be constructed "most economically" in orbit, and would also require a con-

siderable amount of repair and refit technology for their successful long term operation.

The second type of space technology under discussion involves the processing and production, in conditions of weightlessness and vacuum, of materials which would be almost impossible to produce in terrestrial conditions. The materials would include exotic metal and metal-ceramic alloys, high purity glasses, and large single crystals of semiconductors. The cost involved would, of course, be considerable, but this, says Professor Okhotin, is not the issue. Since the results of the processes would be "unique", he argues, their cost-effectiveness should not be a matter for discussion.

In the Soviet economy, where there is centralised budgetary control and planning, there is, theoretically, no need for any major expenditure to be justified to anyone beyond the higher echelons of Party or Government. Nevertheless, although no costs or estimates of the space programme have ever been published—except for vague hints that the cost of the Lunokhod-bearing Moon probes, for example, is only half that of a manned mission—there has, over the years, been a considerable undercurrent of resentment against the enormous expenditure involved.

Kremlinologists have noticed that a number of 'black' jokes have circulated on the theme of 'pie in the sky', correlating achievements in space with food shortages at home. They note that a 'soft' campaign in the Press is standard Soviet practice in preparing the population for some new government drive or campaign.

This new concentration on the cost-effectiveness of space research seems to suggest that, by forestalling possible criticism, next year's Party Congress will include in its "Theses" for the coming Five-year Plan a considerable extension of the Soviet space programme. □

Reprocessing 'scare' in Britain

by Allan Piper

IF a backbench motion at present circulating around the Houses of Parliament gains sufficient support, the reprocessing of spent nuclear fuels in Britain may well be an issue to come under the scrutiny of the Select Committee on Science and Technology during the next Parliamentary session. The matter came to the fore a couple of weeks ago when some newspapers raised a furore over negotiations which could result in the transport of irradiated Japanese nuclear fuels to Britain

for reprocessing at Windscale in Cumbria.

The Parliamentary moves may well have arisen directly from that publicity. But so far the initial press hysteria has signally failed to provoke any other reaction of consequence, and even the 80 or so Members of Parliament who have already signed the motion—and who, according to some reports, can expect wide ministerial support—have chosen a sensibly judicious response to claims that Britain is in danger of becoming a "nuclear dustbin".

Most informed observers are agreed that such misplaced concern can only have sprung from a complete ignorance of both the probable terms of the eventual contract and the overall picture of the British nuclear industry. The deal, which could be worth £300–500 million to Britain, depends on Japanese agreement to take back an amount of waste equal to that produced by the reprocessing.

It is likely also that the British Government will insist that the contract include a clause releasing British Nuclear Fuels (BNFL), which owns the Windscale plant, from its obligations should it fail to develop a successful technique for vitrifying the radioactive waste.

So far the Japanese have shown no signs of baulking at those conditions, which effectively mean that even after the deal has gone through Britain will be left with no 'extra' waste. She will not in any case have to accept Japanese material unless a safe way of transporting its products is developed.

Moreover, the quantity of material which the Japanese will send to Britain—4,000 tonnes will be delivered to Windscale during the decade following 1979—is hardly significant even by comparison with the 1,500 tonnes already reprocessed annually in the UK. (A considerable proportion of this, incidentally, comes from British designed, first generation magnox reactors operating in Latina in Italy and Tokai Mura in Japan.)

Even if the quantity of Japanese material is considered on its own, the figures are rather misleading to the uninitiated: of the incoming 4,000 tonnes of irradiated fuel only some 3% eventually winds up as the potentially dangerous, waste fission products which have aroused the press. Of the rest, about 95% comes out as depleted uranium—which has virtually no radioactivity—and less than 1% becomes fissile plutonium, itself a useful nuclear fuel.

As both of those latter products have some utility—depleted uranium, which is extremely dense, is fast replacing lead in many areas of technology—it seems likely that the Japanese will willingly reprocess them along with the

OUR politicians complain of public apathy. Meetings held during the last General Election were usually badly attended. In many country districts even the appearance of a Cabinet Minister only produced a handful of listeners, and the platform party often outnumbered the audience. It was surprising to find recently in the Huntingdonshire village of Warboys that when the Parish Council called a meeting to discuss the proposal to turn a clay pit on the edge of the village into a dump for toxic wastes, more than 500 people representing every section of the population, turned up.

Concern for conservation of the environment is usually a middle-class, rather elitist preoccupation, and the mass of the population is either apathetic or hostile. Attempts to save ancient buildings, even those of sufficient merit to be "listed" by the Department of the Environment, may be criticised by local councillors who state that these ruins would be better pulled down and replaced by supermarkets, bingo halls or public conveniences. There is little support from all but a minority for setting up nature reserves. It is generally believed that there is little support for spending public funds on such activities as improved sewage works. The Warboys meeting suggests that this last assumption may be wrong, and that there may indeed be votes in sewage.

The Warboys affair is a complicated story. The London Brick Company operates a small works there, using clay dug out of nearby ground. This has produced a deep pit covering about 30 acres. Such holes are now valuable, as our affluent society finds it increasingly difficult to get rid of many types of waste. London Brick has several other holes, and operates a waste tip in Bedfordshire under the somewhat significant trade name of "Easidispense". The company applied for planning permission to use the Warboys pit for the disposal of refuse, including poisonous wastes. This was opposed by the Parish

Council, but it was overruled by the County Planning Committee which gave planning permission, though only under stringent conditions aimed at protecting the local environment.

The people of Warboys were not satisfied. Like other citizens of the old county of Huntingdonshire, which was engulfed in Cambridgeshire on April 1, 1974, they were suspicious of the new authority, and complained that they were being used as a dump—no similar scheme existed in Cambridge-

Votes in sewage?



KENNETH MELLANBY

shire, which sent its toxic wastes to Pitsea in Essex. Notwithstanding assurances from the county staff, they were not convinced that the dump would be safe. The information provided by London Brick about the substances to be dumped gave them little reassurance, for the list resembled the answer to a parliamentary question prepared by a wily civil servant, in that it was true but at the same time said almost nothing. It gave full details of comparatively harmless chemicals, and then such meaningless information as "sludges from wash-pits" and "heavy metals".

The meeting was addressed by county staff and London Brick officials. I was myself involved at the request of

the Parish Council. Having heard of all the precautions to be taken, I asked the company's spokesman if he could give an assurance that the tip would never harm the environment of Warboys. I added that there was cause for concern, as I had visited the Bedfordshire Easidispense dump, having been told by villagers living nearby that it made their life a misery. I said that the dump stank, had poor security and so was a danger to trespassing children—would that at Warboys be better? Very honestly, an official of London Brick refused to give me the assurance I sought. He also reproved me severely, saying that he was surprised that someone of my standing had not approached his company so as to be shown what really went on in its dump. This brought the house down.

For three hours the villagers questioned the officials and the company's representatives. Although the answers were frank, and answered many of the specific worries, the audience remained hostile. When the Chairman of the Parish Council put the matter to the vote, not one single citizen supported the motion that they were satisfied with what they had heard. They all agreed to continue their opposition to the establishment of the dump.

There are lessons to be learned from this affair. We all want to get rid of our waste—but only in some one else's parish. Are we doing enough to reduce the problem, and should we be recycling more materials even when this does not seem to be economic? The main lesson, however, is that the ordinary people *are* concerned about some factors in their environment. If those of us who have been involved with the less popular topics such as old buildings and wildlife can show that we are equally concerned with mundane issues like refuse dumps, then we may be able to recruit a much wider section of the population into the conservation movement. We need them if we are to preserve our, and their, environment.

waste itself. So, all in all, there will be very little foreign material remaining in the "dustbin".

There are other reasons, quite apart from the statistics, why Britain should wish to undertake the reprocessing of foreign nuclear fuels. First, there is a strong political argument. The Japanese approached BNFL in the first place because their own government will grant licences to operate nuclear plant only if provision can be made to reprocess irradiated fuel. Like virtually every other developed nation Japan is now committed to the development of nuclear power as an energy source, at least for the next

couple of decades.

In those circumstances there is really very little reason why Britain, which not only has almost 25 years of valuable and highly regarded experience in the reprocessing field but is also a signatory to the Non-Proliferation Treaty, should discourage a move which ensures that Japan does not develop the wherewithal independently to obtain plutonium and, thus, nuclear weaponry.

There are also sound financial reasons why Britain should continue the negotiations. The fuel which the Japanese hope to send to Windscale will come from American designed

boiling water and pressurised water reactors. Like the latest generation of British reactors, these use uranium oxide rather than metallic uranium. Although the existing facilities at Windscale can reprocess only the latter type of fuel, used by magnox reactors, it is expected that the deal will include a Japanese downpayment of at least £100 million towards the cost of a new £300 million oxide reprocessing plant that Britain will in any case need for her own requirements in the 1980s.

Meanwhile, the final outcome of this latest controversy over what has become something of a hardy perennial in Britain remains to be seen. □

correspondence

Stantec Zebra

SIR,—May we venture to answer your question (October 16, page 544) regarding the present whereabouts of the Stantec Zebra? It is alive, well and working unconscionably hard somewhere approximately 350 miles north of St Albans.

The Computer Board has little reason for complacency as regards the implementation of the Flowers report at Edinburgh Regional Computing Centre (ERCC). Subsequent to the return of a rented IBM 370/158, the centre finds itself without effective access to a major batch processing facility, with concomitant serious consequences for teaching and research at the Universities concerned (Edinburgh, Glasgow and Strathclyde).

The expensive and wasteful debacle ensuing from totally inadequate attempts by the Computer Board to provide comparable facilities on an IBM 370/168 situated at Newcastle University defies comprehension.

Yours faithfully,

D. WHITE	P. MALLISON
C. GILMORE	D. MACNICOL
R. EMANUEL	A. F. CAMERON
K. TYLER	

University of Glasgow, UK

Mosquitoes

SIR,—Though not directly connected with the Research Unit on Genetic Control of Mosquitoes in New Delhi, I have followed closely the progress of its research work and the recent unfortunate controversy which surrounded it. I would like to comment briefly on some of the points raised in the letter (September 18) by Mr C. Raghavan and Dr K. S. Jayaraman of the Press Trust of India on the subject of the research unit:

- The release strategy, as set out in the unit's protocol for the planned experiment in the town of Sonapat, was to adjust the numbers of sterile mosquitoes to be released in relation to the number of wild mosquitoes currently emerging in the town, so that any females unavoidably released would constitute only a small minority of the total female population. The journalists' mention of the release of "at least 2,000 females a day" makes the unwarranted assumption that the unit's maximum possible output of 400,000 males a day would have been required in this experiment. In reality, during the first week of the experiment

35,000 sterile males were scheduled for release daily and these would have been unavoidably accompanied by about 90 females.

- The releases in Sonapat would have been from a grid of over 500 release points in the town. All mosquitoes for release were to have been marked with the same colour. Therefore, among the samples of mosquitoes recaptured there would have been no way of knowing from which of the release points or which day's release they originated. Can the journalists seriously suggest that a specialist in mosquito ecology could 'extrapolate' such data to give information on the dispersal pattern of female mosquitoes which would be of use to someone interested in biological warfare?

- It is typical of the journalists' method of argument that in support of grave allegations against the work of the WHO on mosquitoes in India, they produce an unsubstantiated allegation about the work of the FAO on sugar in Cuba.

- The journalists refer to "bird migration studies supported by the GCMU [sic] and the US Army". In fact the research unit in Delhi had no connection whatsoever with the bird migration study.

- The Assessment of the National Filaria Control Programme (India) 1961–1970 (C. G. Pandit, Indian Council of Medical Research, 1971) recommended a dual strategy based on vector control and drug treatment, contrary to the journalists' statement that exclusive reliance on drugs was advocated. The research at the Filaria laboratories of the Indian National Institute of Communicable Diseases reflects this dual strategy.

- The journalists have missed the point about the conclusion that can be drawn from the history of the eradication of *Aedes aegypti*, but not other *Aedes* species, from Poona. It has never been suggested that this disproves the existence of a degree of cross immunity between dengue antibodies and yellow fever virus. What it does provide is a practical demonstration that if *A. aegypti* (the vector of urban yellow fever) is removed from an Indian town, this does not cause the appearance of yellow fever, contrary to the fears expressed by the journalists about the consequences of the unit's planned experiment in the town of Sonapat.

- I am surprised by the journalists'

opinion that research on "sophisticated technologies" should be restricted to the developed countries, as this runs directly counter to the commonly expressed view that the developing countries require more home-produced technology which has been tested for its feasibility and developed in relation to the actual field conditions in which its application is required. Fortunately the journalists' attitude is not widely shared, and the recent successful genetic control of an *Anopheles albimanus* population in El Salvador (Lofgren, C. S., et al., *Am. J. trop. Med. Hyg.*, 23, 288–297; 1974) suggests that these techniques may provide a practical weapon in the struggle against malaria and other vector-borne diseases on a much shorter time scale than that suggested by the journalists.

Yours faithfully,

R. J. WOOD

University of Manchester, UK



A hundred years ago

MR GLADSTONE AT GREENWICH

WE may surely regard it as a hopeful sign of progress that a whole page of the *Times*, as well as of other daily papers, of last Friday was practically devoted to the discussion of matters connected with Science and Art. When we find the daily papers giving so great prominence to these subjects, and when men of such position and mark as Prince Leopold, Mr Gladstone, and the Lord Chief Justice, take what is evidently a genuine interest in the progress of science and art education among the lower classes, it seems quite safe to infer that this movement has at last taken a prominent and important place in the everyday life of the country.

Prince Leopold, in his address at Oxford, showed that he had taken some pains to become acquainted with the latest statistics of the Science and Art classes. The discrepancy between Prince Leopold's statistics and those of Mr Gladstone has been commented upon in the *Times*. At the same time it is gratifying that a man like Mr Gladstone should think it worth his while to take an interest in the matter at all, but when he does determine to think about it, the least he can do is to inform himself correctly as to statistics.

From *Nature*, 13, 41, Nov. 18, 1875.

news and views

Chromosomal aberrations as tests for mutagenicity

from John R. K. Savage

THE very obvious structural changes which can be induced in chromosomes, and the relative simplicity of the cytological methods which render them observable have made 'chromosomal aberrations' a popular criterion for the evaluation of potential environmental hazards.

Recent developments of staining methods to detect sister chromatid exchanges, SCE (a reciprocal exchange of segments at homologous loci between the two chromatids that comprise the metaphase chromosome arm) have now added another group of structural changes, the induction of which may be utilised as a screening test for mutagens and carcinogens. Results with a wide range of agents reported by Perry and Evans in this issue of *Nature* (page 121) indicate that the test is more sensitive, quicker and easier to use than those employing conventional aberrations. It may also prove to be more relevant since SCE are produced during the DNA synthesis phase, a time when it is thought that most mutations are fixed or established (Auerbach and Kilbey, *A. Rev. Genet.*, **5**, 163-218; 1971).

Perry and Evan's study of SCE illustrates a number of areas where there is a need for caution if the occurrence of induced aberrations is to be used as an indicator of genetic damage.

Of first importance is understanding the nature and mode of action of the agent used to produce aberrations. The properties of the substances used by Perry and Evans covered a wide spectrum. Some had a short half-life giving an acute dose, others were continuously active throughout the treatment period, and one other (cyclophosphamide), because it requires metabolic processes to convert it to an active mutagen, is ineffective *in vitro*. Such information is vital for designing the experimental test.

The need for parallel *in vivo* tests is indicated, not only because of metabolic activation but because the body also has considerable powers of detoxification (Williams, *Detoxication mechanisms*, Chapman Hall, London; 1959). It is possible to expose chromosomes *in vitro* to substances in a form, con-

centration and pH which would be impossible *in vivo*. Moreover a range of different cell systems is needed. Inferences on the mutagenicity of maleic hydrazide based solely on work with plant cells would have been most misleading.

Second, there is a need to appreciate the time of action of the agent in relation to the cell cycle. The majority of chemical substances which produce aberrations, although probably able to induce lesions at all stages of the cycle, require that the cell pass through one or more DNA replication phases in order to allow the lesions to mature into structural chromosome changes. At the same time, these substances invariably cause considerable dose-dependent perturbations in, and delay of passage through, the cycle. Factors such as these will determine how soon after treatment and with what frequencies aberrations will be observed in the test cells. Mitotic perturbation can lead to the simultaneous presence of cells in first, second and even third mitosis after treatment, at the time of sampling, and it may not always be possible to distinguish between them (particularly in the presence of a persistent mutagen) or to assess the effects of cell selection and secondary (derived) aberrations. It is clear that no one 'standardised' experimental design will serve for all mutagenic agents.

Undoubtedly the biggest problem encountered in aberration work is that of quantitation. Given accurate classification and recording of aberration types, can an interpretable dose-response curve be constructed?

On the one hand there is the difficulty of determining the effective 'dose' received by the vital targets in the cell. This may or may not bear a simple relationship to the molar concentration added to the environment of the cells, for it is conditioned by such things as the nature of the agent, the form in which it is supplied, its mode of uptake by the cell, and the density and mitotic status of the test cell populations. If the agent is unstable, the difficulties are enhanced.

On the other hand, there must be some unique quantity or 'yield' to set

against dose. Different yields in different cell cycle compartments (see Fig. 2 of Perry and Evans) alert one to the fact that sensitivity to the agent may change as the cell progresses towards mitosis. In passing it should be noted that such changes are not the same for all types of aberrations. Since the cells of a population do not progress at a uniform rate through the cycle, it follows that samples of metaphases, examined at different times after an agent is introduced, comprise mixtures of cells with differing sensitivities and a corresponding variation in observed mean yield is to be expected. The profile of the variation of yield with time of sampling is influenced by many factors, in particular mitotic delay and perturbation (Savage and Papworth, *J. theor. Biol.*, **38**, 17-38; 1973). All known aberration-inducing agents perturb the mitotic cycle, the degree of perturbation differing with dose.

In these circumstances, a unique yield for a given dose does not exist, so that a dose response curve can take a variety of shapes depending on the sampling methods and times used. A great deal more detailed mathematical work on the kinetics of disturbed cell populations is required before any significance can be attached to the form of such curves.

A fact often overlooked when structural aberrations are used as a test system is the very poor resolution they afford in molecular terms. It is all too easy in discussion to couple molecular damage (such as DNA strand breakage or cross-linkage) and observed chromosomal changes, forgetting size differences of many orders of magnitude. Using the new chromosome banding techniques, the loss of a chromatid band (or a major part of it) is about the limit of aberration detection with light microscopy. The Paris Conference 1971, banding norm (*Birth defects*, **VIII**, 7, National Foundation, New York) has 1,200 chromatid bands in the diploid human female karyotype. If we give equal weight to each band and distribute the 5.8 pg of DNA (Abrahamson *et al.*, *Nature new Biol.*, **245**, 460-462; 1973) uniformly between

them, then each band contains 1.61×10^6 nm or 4.73×10^6 nucleotide pairs (a scale model in cotton thread would require ~ 200 m). Thus many significant structural changes could take place within a band and remain wholly undetected by visual means. Equivalence of visible 'breaks' and molecular lesions is therefore of very doubtful value. The rationale for using aberrations is based on the premise, probably valid, that if a substance induces visible change (much of which is lethal to the cell that carries it) it will also induce invisible change having a high probability of transmission and of genetic consequence. Absence of visible changes however does not imply that there are no invisible ones.

Finally, it is worth considering the nature of SCE in relation to conventional aberrations. The former are all reciprocal and symmetrical (that is, they do not give rise to dicentric chromatids and acentric fragments) complete (no loose 'ends') and, within the resolution limits discussed above, the exchange is confined to homologous loci. This is true also of the occasional chromatid interchanges ('quadriradials') found in Blooms syndrome where SCE is much elevated (Chaganti *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4508-4512; 1974). By contrast, conventional chromatid aberrations, whether produced by chemicals or radiation, are frequently asymmetrical,

often incomplete and certainly not confined to homologous loci either within or between chromatids (Savage *et al.*, *Chromosomes Today*, **4**, 267-276; 1973).

That SCEs constitute a highly sensitive test, and that agents differ in their ability to induce them is now established beyond reasonable doubt, but in the light of the above differences it would seem unwise to substitute them completely for tests using conventional aberrations until more is understood of their relative genetic significance. Both would seem to be necessary at present.

The observations that with certain treatments, chromosomes may be 'shattered' into small discrete segments (Lovelace, *Genetics*, **38**, 675-676; 1953), that chromosomes precociously condensed in S phase appear to be fragmented (Johnson and Rao, *Nature*, **226**, 717-772; 1970) and the increasing evidence that DNA/protein subunits of uniform size exist in eukaryotic chromosomes (McGhee *et al.*, *Chromosoma*, **52**, 189-205; 1975) lead one to speculate on the doctrine of chromosomal integrity during interphase. Might it be that enhanced SCE implies some interference with a mechanism which links chromosomal subunits? Further studies of these aberrations may throw some new light on the vexing question of chromosome structure and organisation. $\square$

nections in the tectum in precisely the same order as before, so that the unfortunate amphibian's visual world is rotated by the same number of degrees as the eye, as his misdirected reflexes bear witness. More recent experiments have done much to explain when and how the retinal cells acquire the information that enables them to re-connect in this invariant way.

The principle of these experiments is to change the position or the orientation of the eye at various stages of embryonic development and to use the retino-tectal map of the adult (mapped electrophysiologically) to assay the effects of the operation. Thus by rotating the eyes of *Xenopus* larvae at different stages, Marcus Jacobson (*Devl Biol.*, **17**, 202; 1968) made the important discovery that the cells acquire fixed positional labels along orthogonal axes which are specified at slightly different stages of development. The antero-posterior axis is irrevocably determined by stage 29; the medio-lateral axis a little later at stage 30 or 31.

More recently, Jacobson and Hunt have found evidence (*Proc. natn. Acad. Sci., U.S.A.*, **20**, 507; 1973) that the information for specifying these coordinates derives from a positional signal operating over the entire animal. They have transplanted eyes to the animals' flanks until after the critical stages and then replaced them in the orbit, to show that the retino-tectal map is normal provided that the orientation of the eye was unchanged with respect to the major axis of the animal during its sojourn in the flank. These results conform to modern theory in the sense that the positional signal apparently conveys only general spatial information and differentiation depends on the cell's interpretation of it.

But how specific is that interpretation? Sperry originally postulated two sets of precisely matching chemical labels in retinal and tectal cells. The experiments of Michael Gaze and his collaborators over the past few years however have shown that if such labels exist at all they must remain labile. The most compelling recent evidence is based on the geometry of retinal and tectal growth. The retina grows in concentric rings, whereas the tectum extends backwards from its anterior lateral pole. In spite of this disparity in the geometry of growth at the two surfaces, the orderly array of neural connections is maintained throughout the different stages of larval development (Gaze *et al.*, *Proc. R. Soc., Lond.*, **B185**, 301; 1974). Gaze and his colleagues suggested on the basis of their observations that the connections continually shift during development, and their suggestion has now been confirmed (Chung *et al.*, *Proc. R. Soc.*

Organising neural development

by Miranda Robertson

IN the experiments of Chung and Cooke, reported on p. 126 of this issue, two lines of enquiry converge on the optic tectum of *Xenopus*, the African clawed toad. The consequence is that the classical embryological concept of specialised organising regions in development can for the first time be brought to bear directly on the specific question of how ordered neuronal connections are set up in the central nervous system.

Embryonic organisers, according to Spemann, who developed the concept in the early years of this century, are specialised regions which control the morphogenesis and differentiation of the tissues immediately surrounding them. The classical organiser has recently undergone a renaissance in the light, in particular, of the positional information theory due to Lewis Wolpert, and it now emerges as the source of a morphogenetic signal providing information on the relative positions of the cells in the developing field. At the earliest stages of development, the entire embryo is a single

field within which the positions of cells are defined by one organiser. According to positional information theory, the subsequent development of the embryo depends on its interpretation of the positional signal. As part of the process of differentiation, the cell acquires a permanent positional label: if it is moved after that stage, it will differentiate in a manner appropriate to its original position.

There is however a stage before the cell's positional label becomes permanent during which the tissue as a whole retains the ability to regulate. If some of the cells are removed at that stage, the remainder adjust to form a complete tissue (or at very early stages, a complete embryo).

It is within this framework that neurobiologists have been attempting to understand the development of the amphibian retino-tectal projection. It has been known since Sperry's classical demonstration in the early 1940s that rotation of an amphibian's eye after section of the optic nerve is followed by the re-establishment of optic con-

Lond., **B187**, 449; 1974).

Clearly there is no unique cell-to-cell matching, and Chung promulgates the view (*Cell*, **3**, 201; 1974)—credited to Lettvin—that what the retinal cells possess is information on their position relative to each other. If that is rigorously preserved, all that is needed at the tectum is an axial polarity to orient the entire array of retinal fibres.

It is the development of that axial polarity that Chung and Cooke have now subjected to experimental scrutiny. The aim of the experiments was to rotate the tectum at different stages of embryonic development and by later mapping the retinal projection, to determine the stage at which its axial polarity became fixed—in a manner precisely analogous to Jacobson's experiments on rotation of the retina.

The operation is technically very difficult because of the size and fragility of the embryonic brain and because at the stage at which most of the operations were performed (21–24) the different brain regions are determined but not yet anatomically distinct. The rotated tissue in each case was of the order of 15 cells square, and in the light of later anatomical analysis, seems sometimes to have included part or all of the adjoining diencephalon, and/or to have excluded part of the tectum.

Anatomically, therefore, the consequences of the operation varied considerably. Because the brain regions at this stage are determined but not yet differentiated, disturbed fragments of diencephalic or tectal tissue regulated to produce complete structures, giving rise to duplicated diencephalons or tecta, or both, at various positions depending on the exact origin and extent of the rotated tissue.

The effect of the anatomical chaos upon the retino-tectal map however turned out to be strictly independent of the orientation or morphology of the tectal tissue itself. The electrophysiological results clearly showed that the axial polarity of the map was determined by the diencephalon. Where the diencephalon was in its normal position anterior to the tectum, the map was normal; where it was transposed so that the tectum was anterior to the diencephalon, the map was reversed. In one case, where a tectum was wedged between two diencephalons, two maps with opposite polarities were recorded, one starting from each tectal edge. Morphological aberrations were ignored by incoming optic fibres. In an animal in which two tecta had developed side by side, the optic nerve entered aberrantly between the tecta but nonetheless spread out over the surfaces of both to give a normally ordered and polarised map.

Chung and Cooke infer from these

Glassy polyethylene

from P. D. Calvert

IN principle any crystalline material can be converted into a disordered glassy solid if it is cooled sufficiently rapidly from the molten state. For some time it has seemed that polyethylene crystallised too quickly for the glassy state to be reached even by splat cooling techniques. But recently Hendra and co-workers have reported the preparation of glassy polyethylene simply by dropping a foil-covered film of polyethylene at its melting point into liquid nitrogen (*J. Polym. Sci. Lett.*, **13**, 365; 1975). The infrared spectrum shows it to be amorphous at 120 K and crystalline after warming to 180 K. Other workers must apparently have allowed their samples to warm up before studying them.

There is no shortage of polymers which can be produced in both crystalline and glassy states but polyethylene is by far the most studied synthetic polymer and there is already controversy over its glass transition temperature. As normally prepared polyethylene is semi-crystalline containing 30–70% amorphous material dispersed between small crystallites. The glass transition temperature of this amorphous phase has been claimed to be 125, 193 or 240 K. In a 1973 review (*Macromolecules*, **6**, 288) Boyer argued strongly for 193 K. Hendra's experiments show crystallisation occurring above ~ 170 K suggesting that the

glass transition must be below this.

At the Shrivenham meeting of the polymer physics group of the Institute of Physics, Hendra discussed some even more controversial results. Using a peak in the Raman spectrum at about 20 cm^{-1} , which is a longitudinal acoustic mode of the whole chain segment within a crystal, they could measure crystal thicknesses. Crystals which form at 160–180 K have a thickness of about 170 Å parallel to the chain. This value is similar to that found in high temperature crystallisation. Since chain motion must be very restricted so close to the glass transition this result suggests that large scale untangling is not necessary for crystallisation and so throws doubt on the chain-folded crystallisation concept which is the basis of most present theories of crystallisation.

Worse still, they found that the crystal thickness formed on warming the glass depended on the temperature at which the polyethylene was melted before quenching to the glassy state. Melting at 400°C gave rise to 155 Å crystals; at 160°C the crystals formed were 180 Å thick. This is heretical in that liquids are not thought to contain any significant structures which could be quenched in and affect subsequent crystallisation. The results might be attributed to degradation of the polymer or destruction of nucleating particles but melting at 160°C subsequent to melting at 400°C again produces 180 Å crystals so the effect is reversible.

results that the diencephalon acts as an organiser controlling the axial polarity of the retino-tectal projection, and preliminary results from grafts of diencephalic tissue alone seem to support this conclusion. They also raise the question of whether the tectal cells ever acquire permanent positional labels.

But the results raise numerous other important questions which are now accessible to experimental investigation. One of the most fundamental concerns the nature of the diencephalic signal. Morphogenetic signals have so far proved uniformly elusive, but until now, neurologists have not known even where to look for them. A further fascinating question is whether it is the same signal that first determines the morphological polarity of the embryo and later provides the axial polarity that guides the optic fibres. The morphological determination of the brain, the determination of axial polarity in the retina, and the determination of the axial polarity of the tectal map are

all clearly separated in time.

But the two known organising regions in *Xenopus* are very close neighbours in space. The diencephalon develops from a region overlying the blastopore lip, which is the classical organiser for the early stages of development.

Could the classical and diencephalic organisers control all three developmental processes with the same signal, interpreted by the cells in different ways at different times? The only evidence so far for temporal changes in the interpretation of an organising signal comes from work on the cellular slime mould (Rubin *et al.*, *J. Embryol. exp. Morph.*, **33**, 227; 1975), which has the heuristic disadvantage that it is not an embryo but the unique advantage of possessing a chemically identified morphogenetic signal. The identification of an organising region in the amphibian brain offers the opportunity of investigating this question in the developing nervous system of a vertebrate. □

Thymus-dependent lymphocytes

from P. B. Medawar and Elizabeth Simpson

Under the patronage of the Rockefeller Foundation a conference was held at the Villa Serbelloni, Bellagio, Italy, from September 1-6, to consider the functional taxonomy of populations of thymus-derived lymphocytes, and by implication their role in the functional integration of the immune system.

THE subjects under discussion included 'T cell differentiation and subsets of T cells,' 'What do T cells see?' 'Ir control and cell interactions,' 'Suppressor T cells,' 'Idiotypes and networks,' and 'The IXth linkage group of genes in the mouse.'

Although the identification of Ly differentiation surface markers for peripheral T cells (Boyse *et al.*, *Proc. R. Soc.*, **B170**, 175; 1968; Cantor and Boyse, *J. exp. Med.*, **141**, 1376; 1975) was seen as a significant step forward in characterising functionally distinct subsets of T cells, the limited availability of anti-Ly sera is an obstacle to the general application of a system of classification based solely on such surface markers. On the basis of the published information referred to above, and unpublished information provided by several participants (E. Boyse, H. Cantor, P. Beverley, D. Schendel and K. Rajewsky) a tentative classification table was nonetheless drawn up taking into consideration the characteristics of three basic peripheral T cell types, T helpers (T_H), T cytotoxic cells and T suppressor cells ($T_{C,S}$) and early or

immature T cells (T_E). The scheme (Table 1) attempts to bring together a number of older observations with the new data obtained from experiments with anti-Ly sera.

In answer to 'What do T cells see?' it is possible that T cells are triggered by antigen associated with molecules of the major histocompatibility complex (H molecules). Three pieces of evidence are particularly relevant.

(1) In some systems cytotoxic T cells seem to recognise a neoantigen formed by association between hapten-like determinants and H-2K and H-2D molecules (see Lennox, *Nature*, **256**, 7; 1975).

(2) Ly 1^+ helper T cells (which are active in both antibody and cytotoxic responses) probably react to Ia determinants, coded by the I region of the major histocompatibility complex, while Ly $2,3^+$ killer cells respond mainly to H-2K (Lipsky and Rosenthal, *J. exp. Med.*, **141**, 138; 1975; Cantor and Boyse, *J. exp. Med.*, **141**, 1390; 1975).

(3) Several studies suggest that most, if not all T cells have receptors for H antigens (for example, Ford *et al.*, *J. exp. Med.*, **141**, 681; 1975).

For triggering to occur, therefore, the T cell receptor may bind to a complex formed by H molecules and specific antigen. Either, one receptor site may bind the neoantigen, or two separate binding sites (on the same or different molecules) may recognise the self H antigen and the foreign haptenic determinant. More specifically it may be that antigen associated with H-2K/H-2D molecules would preferentially

trigger an Ly- $2,3^+$ killer or suppressor cell response, while antigens linked to Ia molecules would preferentially stimulate Ly- 1^+ helper activity. This model implies that the primary function of T cells is to "screen" self H molecules for the presence of non-self determinants. Further work is needed to isolate the T cell receptor and/or the proposed antigen-H complexes before this hypothesis can be rigorously tested.

The way in which I region immune response genes exert their control over cell-cell interactions in immune responses, and hence over both T and B cell responses to specific antigen, is still a matter for debate. There are at least three interactions which must be considered—macrophage/T, T/T and T/B. A requirement for I region identity for these interactions has been shown in some systems (Katz *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2624; 1973) but not in others (McDevitt *et al.*, in *Genes, Receptors, Signals*, 597, Academic Press, 1974; Heber-Katz and Wilson, *J. exp. Med.*, **142**, 928; 1975; von Boehmer *et al.*, *J. exp. Med.*, **142**, 989; 1975). Genetic analysis of the antibody response of different strains to several antigens, Thy 1, LDH, GL Φ and TGAL has demonstrated complementation groups, indicating that at least two genes are involved (Zaleski *et al.*, *Trans. Proc.*, **5**, 201; 1973; Melchers and Rajewsky, *Eur. J. Immun.*, in the press; Dorf *et al.*, *J. exp. Med.*, **141**, 1459; 1975; Rude and Gunther, *Progress in Immunology II*, **2**, 223; 1974; Munro and Taussig, *Nature*, **256**, 103; 1975). For LDH and GL Φ the two genes are encoded in different I subregions of the major histocompatibility complex, while for TGAL both genes may be in the IA subregion. In the response to GL Φ there is some evidence that both genes are expressed on B cells, whereas for TGAL there is evidence that one gene is expressed by B cells ($\pm$ macrophages) and the other on T cells. The simplest view would be that most T cell interactions with other lymphocytes and macrophages are mediated by various proteins bearing different Ia specificities all of which are under Ir control.

The ways in which T cell suppression controls immune responses are still unclear, but recent work has clarified at least some pathways. It has been suggested that an antigen may have different determinants for initiating help and suppression, but that these determinants must be linked on the same molecule.

One very striking finding has been that in several quite different systems, for example antigen-specific suppression, allotype suppression and con A induced suppression (Feldmann *et al.*,

Table 1 T cell subsets

Character	T_H	Subset* $T_{C,S}$	T_E
Ly phenotype	1	2,3	1,2,3
Ontogeny early	+		+
Primary CMI reaction (to H-2)	"LD" (I region)	"SD" (K,D regions)	
Cytotoxic cells:			
Precursors		+	
Effectors		+	
T/T help			
Non-specific (MLR)	+		
T suppression (probably T/T):			
Specific		+	
Non-specific			+
T/B help: specific	+		
Ia phenotype		+	
Histamine receptor		+	
ATx sensitivity			+

+, Character present.

+?, Character probably present.

*It is not implied that all cells in a given subset exhibit all the characters listed.

†Ly 2 positive.

BIOLOGISTS who joined the recent Zaire River expedition brought back a fine collection of flora and fauna, even if at times they may have felt that the price was rather high. In spite of bothersome details such as malaria, amoebic dysentery, fearsome rapids and mysterious footprints, the report delivered by three members of the expedition to a meeting of the Linnean Society on October 16 indicated a mission largely successfully accomplished.

Conditions were considerably better for the scientists, soldiers and other experts who set out under the auspices of the Scientific Exploration Society, in October 1974, than they had been 100 years before for Sir Henry Stanley and his followers who traced the course of the Congo, as the 2,718-mile long river was then called. This time, as Major J. Blashford-Snell reported, there were no hostile war canoes to face, and the inflatable boats used by the expedition were more serviceable than Stanley's Lady Alice, which had to be carried overland whenever rapids were approached. Stanley had no scientists in his party, but this time there were 50, with varied medical, anthropological, geological, zoological and botanical interests. The two latter groups were represented at the meeting by K. A. Joysey (University of Cambridge) and H. W. Woolhouse (University of Leeds), respectively.

Joysey and his colleague, A. Friday, were concerned particularly to

look for specimens of the West African otter shrew (*Potamogale velox*), a large and vicious insectivore that lives in muddy water and is apparently active only at night. After a rendezvous with local pigmies, an otter shrew was eventually found, but proved elusive. The natives later presented the specimen, freshly killed, to the two zoologists who quickly removed the blood needed for sero-taxonomic studies. The body is now in the Museum of Zoology at Cambridge.

Stanley rides again

by Mary Lindley

Other aquatic animals proved less elusive, with local markets yielding a good catch of lung fish (*Protopterus*), elephant fish (various genera of the family Mormyridae) and paddle fish (*Polypterus*) needed for protein sequence studies of muscle aldolase. Throughout the four months of the expedition a party was also collecting fish—from the lakes, tributaries and headwaters—for the British Museum (Natural History). Other ichthyologically inclined members of the expedition collected parasites, bile salts (for an evolutionary study) or fossil specimens. But the entomologists acquired the largest collection. They took home 4,000 Lepidoptera, constituting 800 species, of which a proportion is considered to be un-

described. Experts at the British Museum (Natural History) are now working on these specimens, some of which will eventually be returned to the Government of Zaire. In the tropical rainforest the canopy level turned out to contain the greatest diversity of insect life, which seems to be quite distinct from the insect flora on the ground.

Throughout their journey, the three botanists on the expedition collected seeds of woody legumes for biochemical analysis of unusual amino acids. In southern Zaire, plant and soil specimens were collected for a study of the distribution of plants in relation to copper outcrops and the zonation of the flora was found to reflect the changing concentration of copper in the soil. A few species were growing in unusually toxic concentrations, with some lichens surviving on 5% copper. The material collected will be used in the search for the physiological basis of copper tolerance.

Data for a study of growth and productivity of *Papyrus*, with the aim of investigating the feasibility of making use of *Papyrus* again, was collected from the Upemba swamp basin, where the British botanists found working conditions none too pleasant. Another botanical project was a study of the frequency and distribution of plants which have the C4 pathway of photosynthesis. The outcome of this study will be reported later.

Nature, in the press; Herzenberg and Cantor, unpublished; Cantor and Pierce, unpublished) suppressor cells have an Ly phenotype (Ly 2,3) different from that of helper cells (Ly 1). They also possibly carry histamine receptors (Eichmann *et al.*, *Eur. J. Immun.*, **5**, 511; 1975) and may be Ia positive (Hammerling and Black, *Tenth Leucocyte Culture conference*, 1975). (See Table 1.) The view that at least some T suppressor cells may exert their effect by interaction with T helper cells rather than directly with B cells (Herzenberg and Cantor, unpublished) is supported by much previous data. This may well be a manifestation of helper and suppressor cells belonging to different subsets of T cells having complementary cell surface structures for interaction.

Recent work on idiotypes has shown that an antigen-specific receptor on T cells involved in help for antibody production and on T cells involved in anti-MHC (major histocompatibility complex) responses shares determinants with the immunoglobulin receptor for that antigen on B cells (Binz and

Wigzell, *J. exp. Med.*, **142**, 197; 1975; Eichmann and Rajewsky, *Eur. J. Immun.*, in the press). If this receptor is not cytophilically acquired, this work has important implications concerning the nature of T cell receptors and suggests at least shared expression of a V region gene on both T and B cells, perhaps in association with an H region product (for example Taussig and Munro, *Nature*, **251**, 63; 1974). The fact that the idiotypes of receptors are antigenic and can provoke immune responses (refs above, and Jerne, *Harvey Lecture*, 1975, in the press; Lee *et al.*, *Nature*, **247**, 55; 1974) also opens up the possibility that an immune response to one antigen, once initiated, can provoke another immune response which limits or enhances the first response. This anti-idiotypic response may, in turn, give rise to an anti-anti-idiotypic response, and so on. This network theory has found favour amongst theorists and the data now accumulating suggest that anti-idiotypic responses can occur during an immune response — but there is no good evidence as yet for their having a

normal regulatory role.

The MHC plays an important part in cell-cell interactions during the induction of a whole range of cellular and humoral immune responses. In mice the MHC (H-2) is a series of loci mapping in the IXth linkage group (chromosome 17). Several other possibly analogous loci also exist on this chromosome. Of these the T/t region near the centromere has long fascinated developmental biologists because it governs a sequence of distinct steps in embryogenesis. These events are precisely defined by the recessive-lethal T/t alleles because each of them halts development at the very point where the functional allele would normally act. The prevailing supposition that morphogenetic processes of this kind must involve genetically programmed sensory devices of the sort implied by terms like "cell-cell recognition" is substantiated by the finding that the T/t genetic system, and at least one functional allele (+^{t12}) determines a distinct antigen (F9) that is expressed selectively on cells in a particular phase of differentiation. There

seems to be a reciprocal relationship in the expression of this F9 antigen and the H-2 antigens—F9 in the embryo and H-2 in the adult. The H-2 region lies close to *T/t* and is frequently tied to it in wild mouse populations by the presumably advantageous suppression of crossing over which is a property of *t* alleles. It is therefore of interest that genes at four loci within a region of some 15 centimorgans on chromosome 17 — *T/t*, *H-2K*, *H-2D* and *Tla*—have been shown to specify membrane glycoproteins of similar structure including the incorporation in each of them of a small subunit which is probably β -2-microglobulin. This was the main argument advanced by Artzt and Bennet (*Nature*, **256**, 545; 1975) in support of the hypothesis that this family of four loci are descendants of an ancestral locus that acquired the property of cell surface perception as a condition of metazoan evolution. The immune system can therefore be considered as a special case of adaptive differentiation using as its cornerstones genes which may be reduplications of genes coding for receptors that are important in more primitive cell recognition.

We are grateful to the Wellcome Trust for a contribution to travelling expenses. □

Slugs and plants

from Robert M. May

MUCH information about the natural history of plants and animals may be assembled in an orderly framework by noting that the world tends to provide a continuum of environments, ranging from the unpredictable (where evolutionary pressures are for rapid population growth to exploit the transient good times), through to predictable and biologically crowded environments (where evolutionary pressures are to be a good competitor). These notions, which reach back to Darwin, are commonly formalised in terms of the extremes of "r-selection" and "K-selection", respectively (see *Nature*, **257**, 737; 1975).

One application of these ideas is to the phenomenon of succession. This phenomenon, whereby untended open space tends to revert to weeds, then shrubs, and eventually to woodland, is familiar to all possessors of suburban lawns. The temporal procession from early succession to late succession and climax is a paradigmatic example of a gradient from r-selection to K-selection. On a small scale, such gradients from r- to K-selection have been documented in populations of

dandelions (Gadgil and Solbrig, *Am. Nat.*, **106**, 14–31; 1972), goldenrods (Abrahamson and Gadgil, *Am. Nat.*, **107**, 651–661; 1973) and other wild flowers growing in patches subject to significantly different degrees of disturbance. McNaughton (*Am. Nat.*, **109**, 251–261; 1975) has recently supplemented this work with a meticulous study of populations of cattails (*Typha*) in a variety of sites with differing lengths of frost-free growing periods. He shows the relatively unpredictable locations with a short growing season favour r-selected genotypes which have faster development, produce more offspring (seeds), but invest less energy in each offspring (smaller seeds) and put less premium on traits conferring competitive ability, such as height (to shade out other plants).

A somewhat deeper test of the ideas comes from considering the relation between plants and the beasts that eat them, as a function of successional stage. Compared with late successional plants, those in early succession should invest relatively more of their resources in growth (and reproduction), and relatively less in defence against herbivores. This is so, both for the reasons outlined above, and because the patchy and unpredictable distribution associated with early succession itself provides a degree of escape from herbivores. Thus early successional plant species should provide better food sources for generalised herbivores than do later successional and climax plants. Cates and Orians (*Ecology*, **56**, 410–418; 1975) have recently tested this by taking 100 plant species, of three growth forms and from different seral stages, and determining their short-term palatability to two slug species, one native to western Washington (*Ariolimax columbianus*) and one introduced from Europe (*Arion ater*). Palatability of a given test plant was measured as the ratio of test material eaten to control material eaten, in a well-defined laboratory setting. The results showed that early successional annuals were significantly more palatable than early successional perennials, which in turn were significantly more palatable than later successional species. This validation of theoretical expectations was clear cut, in that no correlation was found between palatability and evolutionary association of the herbivores with particular plant species.

Specifically, Cates and Orians define a "palatability index" as the ratio of log (amount of test material eaten) to log (amount of control material eaten). Then for the native slug *Ariolimax*, the average palatability index for early successional annuals and biennials was 0.96, for early successional perennials

0.77, and for later successional and climax plants 0.46. The corresponding figures for the European slug *Arion* were 0.99, 0.69, 0.40.

A more detailed field and laboratory study of one particular slug-plant system has also been carried out by Cates (*Ecology*, **56**, 391–400; 1975). Using wild ginger (*Asarum caudatum*) and again the native slug *A. columbianus*, he showed the plant populations were polymorphic for growth rate, seed production, and palatability to the slug. In habitats where slugs were relatively rare, populations of wild ginger were dominated by individuals allocating more energy to growth rate and seed production, and less to the production of biochemicals that slugs find nasty. Conversely, in habitats where the slugs were abundant, the more K-selected individuals predominated.

Cates and Orians note that slugs are not "representative" generalised herbivores, because they tend to avoid grasses. Nonetheless, the successional trends they have documented march with the results of other studies, and indicate "broad patterns in the ways plants and herbivores have mutually influenced one another's evolution."

Biochemical factors in schizophrenia

from Richard Rodnight

THE paper by A. G. M. Pearson and A. J. Turner in this issue of *Nature* (page 173) bridges two areas of research—transmethylation and folic acid metabolism—both of which have potential significance for studies of the biochemical basis of psychotic behaviour in man. A disturbance of transmethylation has often been proposed as a possible factor in the aetiology of schizophrenia, but until recently there were few clues as to the pathways which might be involved. A possible mechanism came to light with the discovery in 1961 by J. Axelrod (*Science*, **134**, 343) of a methyltransferase enzyme in rabbit lung that transfers methyl groups from S-adenosylmethionine (SAM) to the naturally occurring indoleamine tryptamine to form mono- and dimethyltryptamine (DMT). The latter compound has been known for many years to induce in normal subjects a model psychosis when administered parenterally, an observation which evokes the suggestion that some of the typical symptoms of schizophrenia may be due to the aberrant production of endogenous DMT in the affected individuals. There is indeed good evidence now that the body does convert minute fractions of its stores of

KARL VON FRISCH'S Nobel Prize winning discovery of the dance language of honeybees is one of the classics of biology. Over many years von Frisch collected evidence to show that when a successful foraging bee returns to the hive it is able to recruit other workers by communicating the precise distance and direction to the food source, encoding this information in the abstract symbolism of a 'dance' performed on the hive surface. The distance is encoded in the speed of dancing and the direction by the orientation of the figure-eight dance in relation to gravity. This remarkable feat places honeybees second only to man and perhaps chimpanzees in the use of abstract symbols in transmission of information.

About eight years ago, Adrian Wenner pointed out that two of von Frisch's best known experiments were not as convincing a demonstration of the dance language as most people had thought. In these two studies, known as the 'step' and 'fan' experiments, von Frisch showed that naive recruit bees would go specifically to experimental food dishes indicated by the dances of experienced foragers, and ignore identical nearby control dishes. In the fan experiment the control and experimental dishes were at the same distance from the hive but in different directions, and in the step experiment the dishes were in the same direction but at different distances. Wenner correctly pointed out that not only is the experimental dish in these experiments unique in being the one indicated by the dance, but also it is the one towards which experienced foragers are flying. The recruits could either be using the dance information or simply watching where the foragers go. Wenner eliminated this confounding variable by training equal numbers of foragers from a separate (control) hive to go to each of the control dishes, and he now found that recruits from the experimental hive no longer preferred the dish indicated by the dance. Wenner's conclusion from this and

other experiments was that recruits may be stimulated to leave the hive by the dance, but that they normally search for food by smell, using the smell of the dancing foragers as a guide.

Naturally enough, Wenner's results aroused enthusiastic criticism, followed by new experiments supporting

The bee language controversy

from John R. Krebs

von Frisch's story, with further counterclaims by Wenner and his colleagues, culminating in a headline in *Nature* (241, 171; 1973) suggesting that von Frisch's data could be explained without recourse to the dance language theory. One valuable consequence of Wenner's criticisms was that people devised more rigorous tests of bee dance communication, and the latest of these studies, by J. L. Gould (*Science*, 189, 685; 1975) finally shows beyond doubt that bees use the dance information.

In a most beautifully ingenious experiment, Gould exposed recruit bees to conflicting information: the dance indicated one direction, while the foragers that gave the dance flew in another direction. The key to Gould's experiment is the fact that bees can be persuaded to orientate their dance in relation to the Sun or (in the case of Gould's experiment) a bright light instead of gravity. As long as the recruits can also see the Sun or bright light, they automatically 'read' the dance correctly, using the light source instead of gravity as a reference. What Gould did then was to cover the ocelli of the dancing foragers with black paint so that they could not see the artificial Sun (a 650 watt bulb) shining into the hive but could still forage and dance. The returning foragers now oriented their dance with respect to gravity, as

usual in a dark hive, but the recruits interpreted the dance as though it were oriented with respect to the bright light. To give an example, suppose the bright light is placed at 60° to the vertical, and a returning forager has just come from a food dish directly in line with the real Sun outside. The forager dances on the vertical surface of the hive, unaware of the bright light, and orientates her dance vertically upwards (which is the gravity-based code for 'towards the Sun'). The recruits, however, read the information as '60° from the line of the Sun', and head off in a different direction from the foragers. Recruits always arrived at food dishes in the direction indicated by their interpretation of the dance, and not at the experimental dish used by the foragers. Further, by moving the bright light to different positions in the hive, Gould could get the recruits to go to different feeding dishes—always the one predicted by incorrect reading of the dance.

It is also particularly satisfying that Gould was able to show at least one reason why Wenner did not find any effect of the dance on recruits. In the initial part of any dance experiment, a few chosen foragers are trained to fly to a particular dish, this being the dish they indicate to naive recruits in the dance. Wenner used training dishes scented with the same odour as was later used in the experiment, while Gould was careful to change to a new scent on the day of the experiment. If Gould used the same scent for both training and experiment, he got the same results as Wenner: the recruits went to any dish with the same scent as that carried into the hive by foragers. The conflict between the von Frisch and Wenner results is at least partly due to details of experimental design and, as Gould points out, this might well mean that recruit bees use the dance information when they are just starting to exploit a new food source but use scent, as Wenner proposed, when exploiting the same food source at a later stage.

indoleamines to N-methylated products with hallucinogenic properties. Traces of both the mono- and dimethylated derivatives of tryptamine and serotonin are excreted in human urine and may occasionally be found in blood; the excretion, moreover, does not seem to be derived from the diet or bacterial action in the bowel. There are also reports, as yet unconfirmed and inadequately documented, that mentally ill patients with the diagnosis of non-affective psychosis tend to

excrete more DMT in their urine than do mentally ill patients with other diagnoses or normal subjects. It is, however, uncertain how much, if any, of urinary DMT is derived from tryptamine methylated in the brain. There is no doubt that human lung contains an SAM-dependent methyltransferase which forms DMT from tryptamine, the enzymic basis for the same reaction in mammalian brain is now much less clear than formerly suspected. Earlier reports on the

occurrence of an indoleamine methyltransferase in brain using SAM as methyl donor were based on unsatisfactory methods of product identification and the reaction urgently needs reassessing by more precise methods such as gas chromatography and mass spectrometry.

Suspensions that folic acid might be involved in the endogenous formation of hallucinogens in the body arose about 3 years ago when several reports appeared indicating that besides SAM,

N⁵-methyltetrahydrofolic acid (5-MTHF) could act as methyl donor for the N-methylation of biogenic amines in the brain; dopamine, apparently yielding its N-methyl derivative epinephrine, appeared a particularly good substrate but it was also claimed that both tryptamine and serotonin could be N-methylated in this system. Unfortunately product identification in these experiments was no better than in the parallel studies using SAM as donor. The usual procedure has been to incubate a crude mixture of soluble proteins from the tissue under study with radioactive SAM or 5-MTHF in the presence of acceptor substrate and then to extract the labelled products with solvents and fractionate them on one or more TLC systems, comparing migration rates with those of authentic compounds. When the combined method of gas chromatography/mass spectrometry was applied by several workers to the products produced in the 5-MTHF-dependent reaction it became evident that methylation of the side-chain amino groups in the case of either dopamine or the indoleamines does not occur. With dopamine as acceptor the product was shown to be a tetra-hydroisoquinoline alkaloid (Laduron and Leysen, *Biochem. Pharmacol.*, **24**, 929; 1975) and with the indoleamines formation of the corresponding tetrahydro- β -carbolines was demonstrated (Wyatt *et al.* *Science*, **187**, 853; 1975). Further examination of the nature of the reaction showed that it consists essentially of the enzymic formation of a 1-carbon compound from 5-MTHF followed by non-enzymic condensation with amine and cyclisation to give the products mentioned above. The 1-carbon unit was shown by Laduron to be formaldehyde, a compound which would be expected to react with aromatic alkylamines by the Pictet-Spengler condensation to give the corresponding cyclic compounds. What then is the mechanism by which formaldehyde is formed enzymically from 5-MTHF? The answer to this question has been given simultaneously by Pearson and Turner (this issue) and R. T. Taylor and M. L. Hanna (*Life Sci.*, **17**, 111-120; 1975). Both groups of workers show that formaldehyde production from 5-MTHF is a result of the activity of the widely distributed enzyme methylenetetrahydrofolate reductase, which is present in crude tissue extracts. This enzyme normally catalyses the NADPH-dependent formation of 5-MTHF from N⁵, N¹⁰-methylene tetrahydrofolate; in suitable conditions and in the presence of electron acceptors it may act in the reverse direction to yield from 5-MTHF, methylene tetrahydrofolate, which in turn is known readily to dissociate into

tetrahydrofolate and formaldehyde. Pearson and Turner have purified the reductase from pig liver by gel filtration and isoelectric focusing and in the process were unable to separate it from the putative dopamine methyltransferase activity. Taylor and Hanna investigated the previously reported stimulation of the enzyme activity by FAD and methylcobalamin (an observation earlier deduced as evidence for an involvement of N⁵-MTHF homocysteine methyltransferase in the N-methylation of biogenic amines) and concluded it is a result of the ability of these compounds to act as non-specific electron acceptors.

While these observations tend to discount a role for folic acid in the endogenous formation of N-methylated hallucinogens it is of interest to note that the β -carboline products bear a close structural resemblance to the alkaloids harmine and harmaline, compounds which reputedly possess psychotomimetic properties in man.

Moreover a case of "folate-responsive schizophrenia" in an adolescent girl has been described (Freeman *et al.*, *New Engl. J. Med.*, **292**, 491; 1975). This patient presented with many of the typical symptoms of schizophrenia and was found on investigation to have a genetically determined deficiency in the activity of the enzyme 5, 10-methylenetetrahydrofolate reductase. The enzyme defect resulted in a decreased formation of 5-MTHF and in homocystinuria due to inefficient remethylation of homocysteine to methionine. The patient's symptoms dramatically improved on administering high doses of folic acid, but reappeared when the therapy was discontinued. It looks as though the complex interrelations of the cerebral metabolism of methionine, folic acid and the cobalamines may remain a fruitful area of research for those workers interested in the role of biological factors in the genesis of the schizophrenic syndrome. □

Angular momenta of planets and asteroids

from David W. Hughes

REGULARITIES in the contemporary structure of the Solar System provide clues not only to the primordial conditions in the system, but also to the accretion mechanisms that formed the planets and satellites and to the subsequent collisions that have taken place between these bodies. The Titius-Bode laws (relating planetary and satellite spacings to their place in the numerical sequence) and the fact that the majority of planets and satellites move in prograde, near circular orbits which lie close to the ecliptic plane are two regularities that have been known for a long time. It is also well known that most planets and satellites spin about axes which are perpendicular to the ecliptic. The relationship between the rotational angular momenta of planetary bodies and their masses is a more recent discovery, however, being first noticed by MacDonald (*Space Sci. Rev.*, **2**, 473; 1963).

The angular momentum of a body is simply the product of its angular spin velocity, ω , and moment of inertia, I . (The moment of inertia is given by $I = Mr^2\alpha$ where M is the mass, r the radius and α the gyration constant; α is 0.4 for a solid equidense sphere). Figure 1 is a logarithmic plot of the angular momenta of planets and asteroids as a function of their masses, the line in Fig. 1 having the form

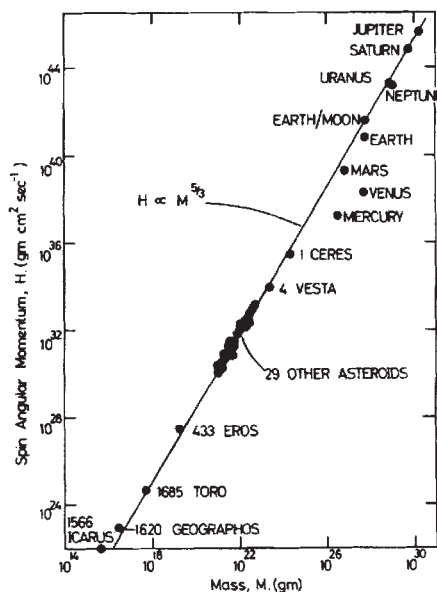
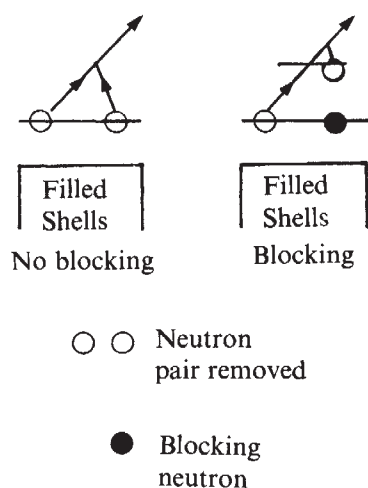


Fig. 1 A logarithmic plot of the spin angular momenta of planets (with the exception of Pluto) and 35 asteroids with well-determined sizes, shapes and rotation rates as a function of their masses (after Fish, *Icarus*, **7**, 251; 1967, Hartmann and Larson, *Icarus*, **7**, 257; 1967 and Burns, *Icarus*, **25**, 545; 1975).

$H = 1.47 \times 10^{-5} M^{2/3}$ where H and M are both in c.g.s. units. T , the rotational period, is found by substituting $T = 2\pi/\omega$ into the above equation. This

NUCLEAR reactions often give us very direct information about the detailed shell structure of nuclei. This is shown particularly well by the many studies of single-particle states by one-nucleon transfer reactions. In these reactions, a nucleon is either removed from a nucleus or given to it, and study of the angular distribution of the outgoing particles tells us the quantum numbers and the occupation probabilities of the single-particle state involved (*Nature*, **245**, 74; 1973; **249**, 695; 1974).

There are also some reactions in which more than one nucleon is transferred at the same time, and these can sometimes tell us about the nucleons that are not participating directly in the reaction. Such



gives $T = 4.3 \times 10^5 \alpha r^2 M^{-1/2}$. If the body is assumed to have a constant density, ρ , throughout (that is, $M = 1.33 \pi r^3 \rho$) and an α of 0.4 then $T = 6.59 \times 10^4 \rho^{-1/2}$, which, when the assumption is made that all Solar System bodies have the same density and no central mass concentration, confirms Alfvén's findings (*Icarus*, **3**, 52; 1964) that all planets and asteroids have approximately the same period of rotation. Using a density of 3 g cm^{-3} this period comes to 8.8 h.

It can be seen from Fig. 1 that Mercury and Venus have considerably lower angular momenta than predicted by the linear relationship. This is due to tidal interactions between these planets and the Sun. The high rate of spin deceleration suffered by Venus indicates that the solid dissipation inside the planet is considerable. This is only possible if large regions in the interior of Venus are near their melting point thus enabling creep, elastoviscosity and direct viscous interactions to play their part in dissipating

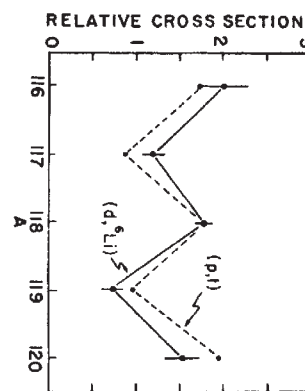
Neutron blocking in alpha-transfer

from P. E. Hodgson

nucleons can get in 'the way, or 'block', the reaction, thus reducing the probability of its taking place.

The first examples of this were found in studies of the (p,t) reaction, which removes two neutrons from the nucleus. In a nucleus with an even number of nucleons both neutrons can be removed from the same single-particle state, whereas if there is an odd number of neutrons it may be necessary to take the neutrons from different states. This latter process is more difficult, and the cross section for the reaction is reduced by a factor of about two. In this way a neutron that does not participate directly in the reaction nevertheless makes its presence felt and thus gives us information about nuclear structure (Fleming, Blann and Fulbright, *Nucl. Phys.*, **A163**, 401; 1971).

This blocking effect has now been observed in alpha-transfer reactions as well. Becchetti and Jänecke (*Phys. Rev. Lett.*, **35**, 268; 1975) have measured the cross sections of several (d, ^6Li) reactions on even and odd isotopes of tin. The reactions on the even isotopes can just remove the two neutrons from one single particle state and the two protons from another, but the reactions on the odd isotopes have to take the neutrons from different states. This is more



Comparison between the relative cross sections of (p,t) and (d, ^6Li) reactions as a function of A for some tin isotopes.

difficult, and as shown in the figure the corresponding cross sections are again reduced by a factor of about two.

This blocking in alpha-transfer is very similar to the same effect in (p,t) reactions, and suggests that the mechanism is essentially the same, with the two transferred protons acting simply as spectators. The regularity of the reduction suggests that it is the $3S_{1/2}$ state that has an odd nucleon in the odd isotopes, and this is expected from the simple shell model.

The spectroscopic factors obtained by analysing the data with the distorted wave theory show a similar reduction for the odd isotopes, confirming that it is not simply due to the differences in binding energies and Q-values, but is a real nuclear structure effect.

energy. Combining the angular momentum of the spinning Earth and the orbital angular momentum of the Moon gives a point on the line in Fig. 1 indicating that the angular momentum of the Earth-Moon system has not changed much during the history of the Solar System. All that has happened is that the Moon has moved further away, and the Earth now spins more slowly.

In a recent paper in *Icarus* (**25**, 545; 1975) Burns doubles the number of asteroid points used in Fig. 1. Accurate sizes based on the radiometry and polarimetry work of Chapman, Morrison and Zellner (*Icarus*, **25**, 104; 1975) are used together with an attempt to include for the first time the effect of an asteroid's non-sphericity. The brightness of asteroids vary with periods scattered around 9 h. These brightness variations, which in some instances range up to two magnitudes but are more typically 0.2 magnitude, are primarily caused by the asteroid having an irregular shape and have been used

by Burns to deduce their shape. As the asteroids usually rotate about their principal axes of maximum moment of inertia they contain more angular momentum per unit mass than a spherical body. The effect of inter-asteroid collisions should make the smallest asteroids spin faster and be more irregular. It can be seen in Fig. 1 that 1566 Icarus and 1620 Geographos both lie above the straight line, indicating that they are spinning faster than expected.

Why is the spin period around 9 h? It seems that the accretion processes which formed the planets and asteroids become less and less efficient as these bodies get nearer and nearer to their rotational stability limit. This limit occurs when the rotational surface velocity of the body is equal to its escape velocity (that is when $T \approx 3.3 \rho^{-1/2} \text{ h}$, a rotational period of $\sim 2 \text{ h}$ for a body of density 3 g cm^{-3}). A conclusion might be that bodies cannot accrete if they spin faster than about one third the rotational stability limit.

Deviations of the spin axes from the normal to the ecliptic are probably due to the statistical fluctuations in the final accumulation of any planet, in other words Uranus' unusual 98° obliquity could be produced by the angular momenta brought in by the last few accreting planetismals. This statistical fluctuation in the accretion process also causes the scatter in the asteroid data.

Two other questions are of interest. First, do these collision-induced spin rates in asteroids ever become so rapid that the surface layers are not gravitationally bound? Excessive spin rates could sweep away any regolith and leave a clean surface. Burns concludes that this does not happen. Even in the case of 1566 Icarus, the smallest and fastest spinning asteroid of known rotation and shape, and 321 Florentina the second most rapid rotator, their densities have to be less than 2.8 and 2.3 g cm⁻³ respectively for this to occur. As asteroids seem to fall into two main compositional groups, however, (Chapman *et al.*, *Icarus*, **25**, 104; 1975) similar to carbonaceous chondrite meteorite (Type I has $\rho=2.5$ g cm⁻³, Type II has $\rho=2.9$ g cm⁻³) and to stony-iron meteorites ($\rho=5.0$ g cm⁻³) things are getting pretty close.

Second, are the spin rates fast enough to cause internal tensile stresses which over long periods of time, split the asteroids apart. Burns again concludes that the answer is no and that if forces are attractive on the surface of the asteroid they are also attractive throughout the interior.

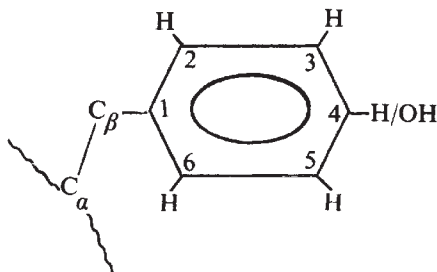
In the past asteroids might have been spinning about other axes than that of maximum moment of inertia. The spin rate would then be faster and these excess rates could have peeled off the requisite outer layers to produce a stable configuration. The fact that carbonaceous chondrite material is very friable and that 1566 Icarus and 321 Florentina are surprisingly spherical for their sizes and rotation rates adds credence to this idea; however more observations of small asteroids are needed to confirm this thesis.

Flipping rings in proteins

from G. C. K. Roberts.

X-RAY crystallography has given us a very detailed picture of the structure of many proteins. This picture is, however, a static one, and in recent years there has been much interest in attempts to understand in detail the dynamics of protein structure. Nuclear magnetic resonance (NMR) spectroscopy is potentially able to give information on the

rate of motion of individual amino-acid sidechains within a protein. The complexity of the NMR spectra of proteins often makes this information difficult to extract, but recently several reports have appeared which focus on the motional characteristics of the aromatic rings of tyrosine and phenylalanine residues.



Sykes and his colleagues have been studying the alkaline phosphatase of *Escherichia coli*, labelled *in vivo* with 3-fluorotyrosine. The ¹⁹F NMR spectrum shows a separate resonance for each of the 11 tyrosine residues per subunit, and a thorough study of the ¹⁹F relaxation behaviour has allowed Hull and Sykes (*J. molec. Biol.*, **98**, 121, 1975) to draw a number of interesting conclusions about the motion of these tyrosine side chains. They observed that the linewidth of the ¹⁹F resonances (related to the relaxation time T₂) was markedly frequency dependent, becoming greater at higher frequency. One relaxation mechanism which would be expected to give rise to a frequency-dependent T₂ is chemical shift anisotropy relaxation. The chemical shift of a nucleus is a tensor quantity which depends on the orientation of the molecule with respect to the external magnetic field. In liquids, the tumbling of the molecule averages the chemical shift over all orientations, but the resulting fluctuations in Zeeman energy provide a relaxation mechanism which is most effective at high fields and for nuclei (such as ¹⁹F) which have a large chemical shift anisotropy. The linewidths of 9 of the 11 fluorotyrosine resonances could be explained in this way, and this analysis led to estimates of the rate of internal motion about the C β -C(1) bond ranging from 10⁶ to 10⁸ s⁻¹ (the overall tumbling of the protein having a rate of about 2 × 10⁶ s⁻¹). For two of the tyrosines, there was an additional contribution to the linewidth from exchange, and for these the rate of reorientation of the ring was slower, in the range 10²-5 × 10⁴ s⁻¹. Analysis of the spin-lattice relaxation time and of the dipolar contribution to T₂ led to an upper limit for motion about the C α -C β bond of 10⁶ s⁻¹.

In favourable circumstances, analysis of the ¹H spectrum of tyrosines and phenylalanines in proteins can give a

rather direct indication of the rate of rotation about the C β -C(1) bond. This bond lies along the symmetry axis of the aromatic ring (see figure). In the free amino acids or simple peptides, the 2 and 6 protons and the 3 and 5 protons are equivalent, and a simple spectrum is observed. For a tyrosine or a phenylalanine in a protein, such a simple spectrum will be observed only if either the environment of the two sides of the ring is identical—an unlikely coincidence—or the ring is rotating sufficiently rapidly for the 2 and 6 and 3 and 5 protons, respectively, to experience an average environment. Roughly speaking, a rotation about C β -C(1) of approximately 200 s⁻¹ will be sufficient to produce this averaging.

In hen lysozyme, all three tyrosine residues show simple spectra indicative of averaging about C β -C(1) (Campbell, Dobson and Williams, *Proc. R. Soc. Lond.*, **B189**, 503-509; 1975). The bovine basic pancreatic trypsin inhibitor has four tyrosine residues, whose resonances have been assigned to individual residues in the sequence (Snyder *et al.*, *Biochemistry*, **14**, 3765; 1975). Three of these show simple spectra at room temperature, while the fourth, Tyr 35, shows a complex spectrum indicative of slow rotation (Snyder *et al.*, *op. cit.*, Wüthrich, presented at *Conference on Molecular Structural Methods in Biological Research*, Stanford, Oct. 9-10, 1975). Similar phenomena are seen for the phenylalanine residues (Wüthrich and Wagner, *FEBS Lett.*, **50**, 265; 1975). Values for the activation energies can be obtained by temperature studies (Wüthrich, *op. cit.*).

It thus seems to be rather common for the aromatic rings of tyrosine and phenylalanine residues in proteins to rotate (the motion is in all probability essentially a flip through 180°) at rates greater than 200 s⁻¹. Some of the implications of this are brought out in a recent paper by Gelin and Karplus (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2002-2006; 1975). These authors have calculated the energy barriers to rotation about C β -C(1) of the tyrosines of the basic trypsin inhibitor, based on its crystal structure, which is known to high resolution. For both the crystallographically determined structure and an energy-refined version thereof, the barriers are extremely high (up to 230 kcalorie mol⁻¹). If the protein structure was allowed to 'relax', however, (simulated by energy minimisation with the tyrosine ring in its minimum and maximum energy orientations), a drastic reduction in the barriers could be achieved at the cost of a rather modest amount of bond angle strain. In this case, at least, it appears that a significant "breathing" of the protein is required to permit the aromatic rings to flip at the rates implied by the NMR experiments.

review article

The protein gap

J. C. Waterlow & P. R. Payne*

The concept of a worldwide protein gap, derived from the diagnosis of kwashiorkor as a protein deficiency state, is no longer tenable. Current estimates of children's protein and energy requirements are considered realistic, and by these criteria the problem is mainly one of quantity rather than quality of food.

"It is a most nourishing and stimulating diet to eat one's own words"
(attributed to Sir Winston Churchill)

IN 1968 the UN Advisory Committee on the Application of Science and Technology to Development presented a report to UNESCO with the title "International action to avert the impending protein crisis"¹. Numerous recommendations were made about methods of increasing protein supplies, the production of high protein foods and the exploitation of unconventional sources of protein to fill the "protein gap". Seven years later there is a strong body of opinion that this is an incorrect statement of the problem: that what the world with its expanding population has to face is primarily a food gap or an energy gap and not a protein gap. McLaren, in his stimulating article "The great protein fiasco"², has summarised the history of this preoccupation with protein and in particular the reasoning by which the UN agencies came to identify protein as the weak point in the world's nutritional defences. It all began with kwashiorkor.

When Williams described kwashiorkor in young children in Ghana some 40 years ago³, she diagnosed it as a nutritional disease, adding cautiously "... in which some amino acid or protein deficiency cannot be excluded". The children who developed the disease did so after weaning, when they were fed on starchy porridges rather low in protein, and they were cured by milk. It took some time for the diagnosis of protein deficiency to be generally accepted; in earlier accounts, particularly from Latin America, the presence of oedema and skin lesions in many cases led to the suggestion of a multiple vitamin deficiency. For the past 25 yr, however, the view has been fairly generally held that the two extremes in the spectrum of childhood malnutrition—kwashiorkor and marasmus—represent conditions in which the main limiting factor is protein on the one hand, energy on the other. This satisfying hypothesis is epitomised in the title of a book published in 1968—*Calorie Deficiencies and Protein Deficiencies*⁴.

The chain of argument which leads from these early clinical studies to the concept of the protein gap as a worldwide problem rests on three premises: that kwashiorkor is indeed a manifestation of protein deficiency; that it is very common in the developing world; and that wherever kwashiorkor occurs milder forms of protein deficiency must be widespread, affecting far more children than those who develop the severe disease. This is the "tip of the iceberg" theory. All three premises need to be examined.

Is kwashiorkor a result of protein deficiency?

The evidence is to a large extent circumstantial. Retrospective dietary histories are of limited value because the illness itself causes a fall in appetite and thus a reduced intake of all nutrients. The most powerful evidence of cause and effect in nutritional work—the therapeutic test—is not practicable, because a sick child cannot be fed protein alone. Brock *et al.*⁵ attempted this approach when they produced "initiation of cure" (loss of oedema) with a diet in which the only source of nitrogen was a mixture of pure amino acids, but they had to give a source of energy as well. Some of the biochemical features of kwashiorkor, notably the low serum albumin and altered amino acid pattern^{6,7}, indeed suggest a state of protein depletion. Nevertheless, our understanding of the pathogenesis of the major features of kwashiorkor, oedema and fatty liver, is still incomplete. It is reasonable to suppose that oedema results in part from the low serum albumin concentration, although not all workers agree. Evidence suggests that the most likely cause of fatty liver is a failure of synthesis of the protein part of the lipoprotein which is responsible for fat transport out of the liver^{8,9}. Animal experiments have also provided useful evidence: the rat is a poor model, but many of the features of kwashiorkor have been produced in pigs¹⁰ and in monkeys¹¹ by feeding a diet high in carbohydrate and low in protein. Grimble and Whitehead¹² found, however, that in pigs the biochemical changes typical of kwashiorkor did not appear until the appetite of the animals had fallen, so that their food intake was reduced. The evidence is therefore not clear cut, but one difference between kwashiorkor and marasmus is undeniable: children with kwashiorkor must have been less energy-deficient than those with marasmus, for the simple reason that their bodies contain more fat, as is immediately obvious at autopsy.

The first serious attack on the conventional theory came from Gopalan in India¹³. In a prospective study of children in a poor community no quantitative or qualitative differences in the diet were found between children who developed kwashiorkor and those who became marasmic. Gopalan therefore suggested that it is not the diet which determines the clinical picture, but the way in which the subject adapts or fails to adapt. One explanation¹⁴ (which does not take us very far) for a difference between children in their response to an inadequate diet lies in the known variability between individuals in their requirements for energy and for protein. One could conceive that on a diet which is marginally deficient in both, a child who happened to have a high energy requirement would develop marasmus, and one with a high protein requirement would develop kwashiorkor.

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The basis for intra-individual variation in requirements is quite unknown. One may speculate a little further. When energy intake is inadequate, protein, mainly from muscle, is oxidised to fill the gap. It has been said that the marasmic lives on his own meat; that is, that the net efflux of amino acids from muscle provides enough substrate for synthesis by the liver of albumin and apolipoprotein, so that the characteristic features of kwashiorkor do not appear. The release of amino acids from muscle, and the extent to which they are utilised for protein synthesis in other tissues or for gluconeogenesis, probably depends on the balance of at least three and possibly four hormones—insulin, cortisol, glucagon and growth hormone. Whitehead *et al.*¹⁵ have described progressive disturbances in endocrine balance in kwashiorkor and there is some evidence of endocrine differences between kwashiorkor and marasmus¹⁶. Again we do not know whether these are effects of distinct causes or whether they represent the inherent variability of response.

Finally, there are differences, which may be important, in the natural history of kwashiorkor and marasmus. It is often said that marasmus typically occurs at an earlier age, but this is not universally true. Within each of the three countries—Jamaica, Iraq and Jordan—for which we have comparable data, there seems to be no significant difference in the age at which the different types of case present at hospital. In all three series, however, the marasmics were more stunted in height than the children with kwashiorkor¹⁷⁻¹⁹. The same has been found in unpublished studies from Sudan and Nigeria. This is clear evidence that marasmus is a more long standing condition and that its onset must have been earlier. It has also been

said that kwashiorkor is a rural, marasmus an urban, disease; and that marasmus is getting commoner because of increasing urbanisation and decreasing breast feeding. These statements are plausible, but they are not supported by firm epidemiological evidence. The reason is not just a lack of adequate surveys, but also the lack of an agreed and standardised system of reporting, without which it is impossible to make comparisons between different regions or to identify changes that are occurring with time. If, as the literature suggests, the prevalence of kwashiorkor differs in different countries with different dietary patterns, there should here be an important clue to aetiology¹⁷. This clue has not, however, been systematically followed up.

Is there evidence of widespread protein deficiency in the absence of clinical disease?

Estimates by WHO^{21,22} that in many countries 20–40% of children are moderately or severely malnourished are in most cases based on a deficit in weight for age. This growth failure is, of course, a nonspecific effect of malnutrition. It is possible that more information may be obtained by analysing separately two components of growth failure: inadequate gain in weight and inadequate gain in length or height^{17,23}.

The search for specific biochemical indicators of protein deficiency has been going on for many years. Whitehead, a pioneer in this field, has concluded that the most sensitive index of impending kwashiorkor is a decrease in serum albumin concentration²⁴. The greater precision of modern automated methods makes it possible to detect small deviations from

Table 1 Estimates of protein and energy requirements at 1 yr of age

Year	per kg body weight			Protein energy Total energy (%)	Source	Reference
	Protein* (g)	Energy† (kcal)	(kJ)			
1948	3.3	100	420	13.2	NRC (USA)	47
1957	2.0	100	420	8.0	FAO	48
1964	2.5	100	420	10.0	NRC (USA)	47
1965	1.1	100	420	4.4	FAO/WHO	49
1968	1.8	100	420	7.2	NRC (USA)	47
1969	1.3	110	460	4.7	DHSS (UK)	50
1973	1.27	105	440	4.8	FAO/WHO	27
1974	1.35	100	420	5.4	NRC (USA)	47

*As protein which is 100% utilised.

†Units of energy as in original publications. Rounded off to 4.2 kJ = 1 kcalorie.

suggested that kwashiorkor is typically precipitated by an acute infection such as measles, whereas marasmus infection for example, gastroenteritis², tends to be chronic and recurrent.

Many readers may regard this discussion of the characteristics of childhood malnutrition as academic and irrelevant to the question of the protein gap, but it is important to realise how much is still unknown about the way in which children adapt or fail to adapt to deficient diets. This lack of knowledge has practical importance.

Is kwashiorkor common?

When the WHO and FAO became concerned about childhood malnutrition after the war, one of the first results was a survey in Africa by Brock and Autret²⁰. Their report concluded that "kwashiorkor is the most serious and widespread nutritional disorder known to medical and nutritional science". McLaren² has pointed out that this judgment, based largely on rural Africa, ignores large areas of the world where marasmus is by far the commonest form of malnutrition. It is also frequently

accepted normal levels that are statistically significant. It remains to be proved, however, that this change is indeed a specific effect of protein deficiency.

The third approach is by comparison of intakes with requirements. Before going on to this we have to consider the basis on which requirements have been estimated, since they are often considered not to be realistic, or little better than guesses.

Estimates of requirements

Table 1 shows how estimates of the requirements of a 1-yr-old child for protein and energy have changed over the years.

Protein requirements. The figure of 1.27 g kg⁻¹ in terms of milk protein is in our view very well founded. It is easier to estimate the requirement of an infant than of an adult because growth provides an extremely sensitive criterion of whether the child's needs are being met. In infants up to about 6 months the requirement is based on the observed intakes of infants consuming breast milk freely and growing normally²⁵. This could even be slightly more than the minimum requirement. At about 1 yr

the requirement has been derived from measurements of the minimum amount of cow's milk protein necessary to secure normal growth²⁶. As might be expected, it is slightly less per kg than that of the younger infant who is growing faster. Of the requirement of 1.27 g kg⁻¹, it is calculated that about seven-eighths is needed for maintenance and only about one-eighth for growth²⁵. We have found that if the protein intake of infants is reduced from an adequate to a maintenance level, with no change in energy, growth falls off within 1 or 2 d. It seems that the amount used for maintenance is so closely fixed that there is no margin to supply the needs for growth if the intake is slightly reduced.

In an adult man the latest estimate of protein requirement²⁷, in terms of egg protein, is 0.57 g kg⁻¹ d⁻¹: about half that of

Table 2 Protein quality of cereals determined in different ways

	Utilisable protein as % of total energy			
	Method of determination			
	NPU (rat)	NPU (child)	Protein score	Average
Maize	4.7	4.8	5.4	5.0
Millet	5.3	5.3	7.7	6.1
Rice	4.9	6.1	6.5	5.8
Wheat	5.9	5.2	5.6	5.6

the 1-yr-old child. This figure is derived from the amounts of protein needed to secure nitrogen balance. For an adult of average weight this comes to about 40 g of milk or egg protein per d, or about 60 g if corrected for the lower protein quality of the average mixed diet. This is about two-thirds of the amount of protein eaten daily by most people in this country. Many people feel strongly that these estimates of protein requirement are unrealistically low, and that the protein gap has been artificially abolished by the stroke of a pen, simply by reducing the requirement. If the estimates for infants are well founded, however, it is difficult to see why those for adults should not be correct. The lower requirement for the adult is not due simply to the absence of growth; the adult is considered to have a lower maintenance requirement, and this makes sense, because the rate of many metabolic reactions decreases with increase in body weight. Examples are the basal oxygen uptake, the rate of total protein turnover²⁸ and the rate of albumin synthesis^{29,30}. Young *et al.* have shown a close correspondence between protein requirements and protein turnover rate over a whole life span, from prematurity to old age³¹.

Protein quality. The protein requirement of 1.27 g kg⁻¹ d⁻¹ is in terms of milk protein, which is almost 100% utilised when fed at or below the requirement level²⁶. In fact, at the age when children develop kwashiorkor they are not fed on milk alone, and so this figure has to be corrected for the quality of the dietary protein. There is much difference of opinion about methods of determining protein quality³², and, in particular, whether biological values determined on rats are applicable to man. In theory, results on the rat should underestimate quality, because the rat is growing faster than the child. Protein quality depends on the proportion and pattern of the essential amino acids. The essential amino acid requirement for maintenance is relatively low, because recycling of these amino acids is extremely efficient^{33,34}. For growth, however, the essential amino acids have to be provided by the diet for deposition in tissue protein. The proportion of essential to total amino acids in the diet must therefore in theory be higher the faster the growth rate. Nevertheless, as Table 2 shows, values for the protein quality (utilisable protein content) of cereals measured directly in rats and in children are in good agreement, and agree also with theoretical estimates based on the proportions of essential amino acids in these proteins (protein scores).

For many purposes it is convenient to convert the amount of utilisable protein provided by a diet or foodstuff from units

of weight to units of energy, and to express it as a percentage of the total energy. This is the *P/E* ratio. The figures in Table 2 are expressed in this way. For comparison, the *P/E* ratio in breast milk (early lactation) is about 7.5%, falling to 5% at 2.5 to 3 months of age³⁵.

Energy requirements. Estimates of the energy requirements of young children have changed less than those for protein (Table 1). The energy requirement is made up of three components—maintenance, growth and physical activity, and we now have fairly reliable figures for each of these. It has been found in a number of animal species, including man, that the maintenance requirement—that is the minimum energy intake at which body weight is constant—is about 1.5 times the basal metabolic rate (BMR)³⁶. In the 1-yr-old child the BMR is 210–230 kJ kg⁻¹, so that the maintenance requirement may be taken as 330 kJ kg⁻¹. Studies in children recovering from malnutrition and gaining weight very rapidly have shown that the energy cost of weight gain is about 40 kJ kg⁻¹ (ref. 37), if it is assumed that the tissue deposited contains normal amounts of protein and fat. This figure includes both the stored energy and the energy cost of synthesis.

More recent work in Jamaica by Spady (unpublished) suggests that this is an overestimate. He obtained a figure of about 20 kJ per g weight gain. This is the lowest yet found, but is still higher than the theoretical cost of peptide bond and fat synthesis. The rate of weight gain in a normal infant at 9–12 months would be about 1 g kg⁻¹ d⁻¹. If we accept the conventional estimate of 430 kJ kg⁻¹ for the total daily requirement, this would leave 80 kJ d⁻¹ for physical activity. Until very recently these components of energy expenditure in infants have not been studied directly. Spady obtained experimental confirmation of the maintenance energy needs of young children with the instrument known as the SAMI, which counts and integrates the total number of heart beats. After calibration in each child it is possible to monitor the total energy expenditure over a whole day without interfering with the child's activities. Very good agreement was found between energy intake from food and the sum of energy expenditure and energy stored during growth. These results suggest that the estimate of the

Table 3 Energy and protein intakes of children aged 1–2 yr in various countries. Individual weighed intakes after weaning

Country	No. of child days	Average intake per kg body weight	
		Protein (g)*	Energy (kJ)
Ghana	30	1.19	360
Guatemala	28	1.16	323
Jamaica	266	1.47	348
Polynesia	72	1.32	295
Thailand	54	0.61	218
Uganda	124	1.28	285

Sources given in ref. 17.

*As protein which is 100% utilised. Utilisation of protein in actual diets assumed to be 60%.

amount expended in physical activity is reasonable, although of course there will be much individual variation.

Perhaps the most important conclusion from this "dissection" of energy expenditure is that the maintenance requirement is relatively so large. An intake of about 320 kJ kg⁻¹, which is quite common in children in developing countries (Table 3) is just enough for maintenance, with no margin for growth or extra activity. Quite apart from the physical effects, this limit on intake may also be bad for the child's mental development, as it will reduce exploration, play and social activity.

Individual variation. The estimates which have been given of protein needs represent what have been called 'safe levels'²⁷. These are amounts sufficient to meet the needs even of individuals having unusually high requirements, and are obtained by adding

2 standard deviations ($\pm 30\%$) to the average requirement. In fact, this estimate of 15% for the variability between individuals is almost certainly too high³⁸, so that the safe levels are very safe.

For energy, on the other hand, there is the complication that the cause of variation is partly metabolic and physiological, because of differences in maintenance expenditure and growth rate, and partly due to differences in physical activity. The only estimate we can make of the first source of variation is from measurements of basal metabolic rate, of which the s.d. may be taken as 6.5% (ref. 39). That of the second component—physical activity—will presumably be much larger. With energy, to add a margin of safety will mean that those who need less get too much, and the figures quoted in Table 1 are therefore simply the average values for groups of children.

Comparison of intakes and needs

The question originally posed was whether there was evidence of widespread protein deficiency in children. It would be more precise to ask whether there is evidence that protein is limiting. If the total food intake is inadequate there will be a secondary protein deficiency, because protein cannot be utilised if energy

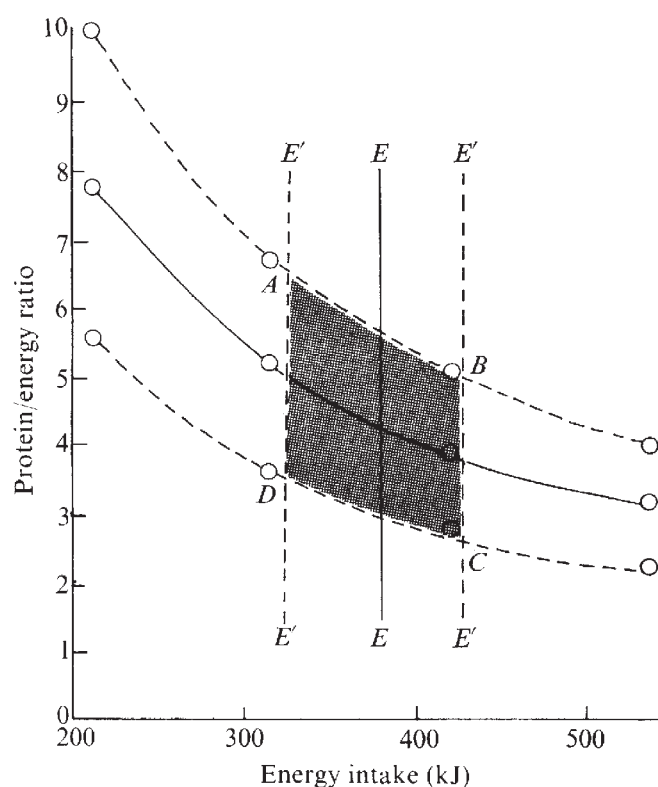


Fig. 1 Ratio of utilisable protein (kJ) to total energy of diets which will provide the average requirement of protein (—) and the average $\pm 2\sigma$ (dotted curves), for a 1-yr-old child. The top curve represents the "safe level". Vertical lines are estimates of the energy requirement for maintenance + growth + minimal physical activity. The solid line is the average, the dotted lines the average $\pm 2\sigma$. The shaded area is the "area of ignorance" resulting from the ranges of individual variation.

is lacking, and extra protein will be of no value. To answer the question, accurate measurements have to be made of the actual food intakes of individual children, and not simply of the family as a whole. Moreover, because of the difficulty of

measuring intakes of breast milk, they must be made on children who have been completely weaned. Studies which fulfil these criteria have been made in a number of countries, but even so, when the literature was summarised two years ago¹⁷, the number of child days covered by the measurements was very small in relation to the importance of the problem. With one exception, all these studies showed that in children 1–2 yr old there was a greater deficit in energy than of protein (Table 3).

Another study in Uganda⁴⁰ showed energy intakes 30% below the recommended level, with a marked reduction in physical activity compared with European children. These results are of particular interest because they were obtained in a district where kwashiorkor has long been considered the typical form of malnutrition.

It is obvious that to prevent malnutrition the energy deficit shown in Table 3 must be made up. It then becomes important to define the kind of diets which will fulfil protein needs when enough is eaten to satisfy energy needs. The figures already given show that at 1 yr the protein requirement, expressed as a proportion of the energy requirement, is 5%, so that in theory a diet with a P/E ratio of 5% should meet the child's needs if enough of it is eaten. The problem, however, is complicated by the individual variability of requirements⁴¹.

Figure 1 shows the P/E ratios in diets which will provide the safe level of protein when eaten at different levels of energy intake. The vertical line $E-E$ is an estimate of the minimum energy requirement for health, and represents the requirement for maintenance and growth, with a very small addition for physical activity. This estimate is an average: we do not know the extent of individual variation, so the best we can do is to assume it to be the same as for the BMR, that is about 6.5% (ref. 39). The lines $E'-E'$ represent $E \pm 2\sigma$ ($\pm 13\%$). The point A represents the "worst" case, of a child who has the highest need for protein and the lowest for energy. The corresponding P/E ratio is 6.5%. Conversely, the child at C has the lowest protein and highest energy need. A and C then represent the extremes of the range of P/E ratios needed if protein and energy requirements are negatively correlated—a very unlikely situation. Similarly, points B and D represent the range of P/E ratios in a population in which the two requirements are positively correlated. B corresponds to a P/E ratio of 5.0%. There could, of course, be intermediate situations with protein and energy needs being more or less independent in a random fashion (zero correlation). Beaton and Swiss⁴² suggested a value of $+0.2$ for the correlation coefficient. This may be too low, if, as in this case, we are discussing a situation in which physical activity, and thus its contribution to the variance, is low. Protein turnover is a process which is expensive in energy and therefore accounts for a substantial part of the basal energy requirement. The demonstration by Young *et al.*⁴¹ of a parallel between protein turnover rate and requirement has already been mentioned. These considerations suggest that there is likely to be a fairly strong positive correlation ($> +0.2$) between individual needs for protein and energy.

All we can say so far is that the safe level for the P/E ratio is unlikely to be greater than 6.5% or less than 5.0%, and more research is needed to fill this gap. This is not academic hair-splitting but of great practical importance. Reference to Table 2 shows that the P/E ratios of cereals fall within the likely range of the safe level. The suggestion that cereals without any supplement might provide safe amounts of protein for weaning children may be unacceptable to many. Begum *et al.*⁴³ have shown that normal growth could be sustained in 2–3-yr-old children by diets in which wheat or rice were the sole sources of protein, but up to now this has not been demonstrated for younger ages. A more precise knowledge of the safe P/E ratio would enable us to assess the significance of the relatively small differences between the cereals; for example, is the somewhat lower value for maize related to the clinical impression that kwashiorkor is commoner in areas where maize is the staple food? The main disadvantage of cereals, and particularly maize, as a principal food for young children is, however, that

their bulkiness when cooked makes it difficult for the child to eat enough to satisfy his energy needs, unless—as is unlikely—he has very frequent meals.

The effect of infections

The estimates of protein and energy requirements proposed by FAO/WHO²⁷ are for healthy people. Even before their latest report was published, its recommendations on protein requirements were described by the Protein Advisory Group of the UN as inadequate, on the ground that no allowance was made for the effect of infections. The argument was that since infections cause a loss of body nitrogen, a high protein supplement is needed to make good these losses. We do not know what is the extent of the nitrogen loss in conditions of real life (as opposed to a metabolic ward). The loss of nitrogen must, however, be accompanied by a loss of energy stores because of the reduced food intake and the extra heat production caused by fever. The extent of these losses is again unknown, but perhaps the problem can be tackled in a different way. The child with an infection ceases to gain weight and very often loses weight. We may then ask, what is the diet necessary for him to catch up and regain his correct weight after the infection is over? If the child has been at constant weight for a month, he needs to gain at twice the normal rate for the next month in order to catch up. Table 4 shows the average *P/E* ratio needed for different rates of catch-up. Whitehead⁴⁴ has published a similar table, using a different value for the energy cost of growth. Even at rather high growth rates, the required increase in

to be done to fill the gap is another story. It is important that scientists in this field should make sure that the pendulum does not swing too far. One difficulty is that the controversy about the protein gap, which represents a genuine difference of scientific opinion, is accompanied by another controversy, potentially far more dangerous. This arises from the attitude that research on these nutritional problems is academic, irrelevant and a waste of time; that we know how to prevent malnutrition and therefore what matters is to use this knowledge. Malnutrition is an emotive subject; it is certainly true that there is much that can be done with existing knowledge, and that action cannot wait on the perfectionism of research. But perhaps the story of the protein gap shows the arrogance of supposing that we know the answers, and illustrates the need for a continuing critical examination of the premises on which action is based.

In the long run, the prevention of malnutrition must be founded on an understanding of how the body adapts to different dietary situations, because otherwise concepts such as "deficiency" and "requirement" have no meaning. This is particularly important in relation to marginal states of malnutrition. Nutrition has been called the "Cinderella of the sciences"—justly so, because like Cinderella it is pulled in two directions.

Table 4 Protein and energy requirements for catch-up growth

	Protein (g kg ⁻¹ d ⁻¹)	Energy (kJ kg ⁻¹ d ⁻¹)	<i>P/E</i>
Maintenance	1.10	440	4.2
Maintenance + normal growth × 1	1.25	465	4.55
Maintenance + normal growth × 2	1.40	490	4.8
Maintenance + normal growth × 3	1.55	515	5.05
Maintenance + normal growth × 4	1.70	540	5.3
Maintenance + normal growth × 5	1.85	565	5.5

Protein increment for normal growth taken as 0.15 g kg⁻¹ d⁻¹. Maintenance requirement for energy includes normal level of physical activity. Maintenance requirement for growth taken as 25 kJ per g weight gain.

protein content is not very great. Whitehead and his colleagues in Gambia (personal communication) have studied the inter-relationship of three factors in rural children: food intake, weight gain and the number of days per month that the children were ill. A higher food intake, derived from the usual diet only, with no supplements, had a very clear effect in counteracting the growth failure caused by infections.

Another stress is pregnancy. In communities where the women are undernourished a high proportion of infants are born with a low birth weight. Work in Guatemala⁴⁵ has shown that increasing the mother's energy intake during the last trimester of pregnancy by a total of 9 MJ or more (as carbohydrate) caused a significant increase in birth weight and a reduction in the infant death rate in the first year of life.

Understanding and prevention

The evidence we have put forward leads to the conclusion that the protein gap is a myth and that what really exists, even for vulnerable groups, is a food gap and an energy gap. This point of view is now fairly widely accepted, particularly in this country, where it has become part of aid policy⁴⁶. What needs

- ¹ United Nations, Report to the Economic and Social Council of the Advisory Committee on the Application of Science and Technology to Development (United Nations, New York, 1968).
- ² McLaren, D. S., *Lancet*, ii, 93–96 (1975).
- ³ Williams, C. D., *Archs Dis Child*, 8, 423–433 (1933).
- ⁴ McCance, R. A., and Widdowson, E. M. (eds), *Calorie Deficiencies and Protein Deficiencies* (Churchill, London, 1968).
- ⁵ Brock, J. F., et al., *Lancet*, ii, 355–360 (1955).
- ⁶ Grimble, R. F., and Whitehead, R. G., *Lancet*, i, 918–921 (1970).
- ⁷ Whitehead, R. G., Frood, J. D. L., and Poskitt, E. M. E., *Lancet*, ii, 287–289 (1971).
- ⁸ Truswell, A. S., and Hansen, J. D. L., *S. Afr. med. J.*, 43, 280–283 (1969).
- ⁹ Seakins, A., and Waterlow, J. C., *Biochem. J.*, 129, 793–795 (1972).
- ¹⁰ Platt, B. S., Heard, C. R. C., and Stewart, R. J. C. in *Mammalian Protein Metabolism II* (edit. by Munro, H. N., and Allison, J. B.), 446–521 (Academic, New York and London, 1964).
- ¹¹ Kumar, V., Deo, M. G., and Ramalingaswami, V., *Gastroenterology*, 62, 445–451 (1972).
- ¹² Grimble, R. F., and Whitehead, R. G., *Br. J. Nutr.*, 24, 557–564 (1970).
- ¹³ Gopalan, C., in *Calorie Deficiencies and Protein Deficiencies* (edit. by McCance, R. A., and Widdowson, E. M.), 49–58 (Churchill, London, 1968).
- ¹⁴ Waterlow, J. C., *Lancet*, ii, 712 (1974).
- ¹⁵ Lunn, P. G., Whitehead, R. G., Hay, R. W., and Baker, B. A., *Br. J. Nutr.*, 29, 399–422 (1973).
- ¹⁶ Rao, K. S. J., *Lancet*, i, 709–711 (1974).
- ¹⁷ Waterlow, J. C., and Rutishauser, I. H. E., in *Early Malnutrition and Mental Development* (edit. by Craviotto, J., Hambraeus, L., and Vahlquist, Bo), 13–26 (Almqvist and Wiksell, Stockholm, 1974).
- ¹⁸ Shakir, A., Demarchi, M., and El-Milli, N., *Lancet*, ii, 143–146 (1972).
- ¹⁹ Hijazi, S. S., *Am. J. clin. Nutr.*, 27, 1254–1258 (1974).
- ²⁰ Brock, J. F., and Autret, M., *WHO Monograph Series No. 8* (WHO, Geneva, 1952).
- ²¹ Bengoa, J. M., *WHO Chronicle*, 24, 552–561 (1970).
- ²² FAO/WHO, *WHO Tech. Rep. Ser. No. 477* (WHO, Geneva, 1971).
- ²³ Waterlow, J. C., *Lancet*, ii, 87–89 (1973).
- ²⁴ Whitehead, R. G., Coward, W. A., and Lunn, P. G., *Lancet*, i, 63–66 (1973).
- ²⁵ Fomon, S. J., *Infant Nutrition* 2nd ed., 118–151, (Saunders, Philadelphia, 1974).
- ²⁶ Chan, H., and Waterlow, J. C., *Br. J. Nutr.*, 20, 775–782 (1966).
- ²⁷ FAO/WHO, *WHO Tech. Rep. Ser. No. 522* (WHO, Geneva, 1973).
- ²⁸ Waterlow, J. C., in *Mammalian Protein Metabolism III* (edit. by Munro, H. N.), 325–390 (Academic, New York and London, 1969).
- ²⁹ James, W. P. T., and Hay, A. M., *J. clin. Invest.*, 47, 1958–1972 (1968).
- ³⁰ Hoffenberg, R., Black, E., and Brock, J. F., *J. clin. Invest.*, 45, 143–152 (1966).
- ³¹ Young, V. R., Steffee, W. P., Pencharz, P. B., Winterer, J. C., and Scrimshaw, N. S., *Nature*, 253, 192–193 (1975).
- ³² Porter, J. W. G., and Rolls, B. A. (eds), *Proteins in Human Nutrition* (Academic, New York and London, 1973).
- ³³ Stephen, J. M. L., and Waterlow, J. C., *Nature*, 211, 978–980 (1966).
- ³⁴ Waterlow, J. C., and Garlick, P. J., in *Alcohol and Abnormal Protein Biosynthesis* (edit. by Rothschild, M. A., Oratz, M., and Schreiber, S. S.), 67–94 (Pergamon, New York, 1975).
- ³⁵ Wheeler, E. F., *Am. J. clin. Nutr.*, 26, 631–639 (1973).
- ³⁶ Payne, P. R., and Waterlow, J. C., *Lancet*, ii, 210–211 (1971).
- ³⁷ Ashworth, Ann., *Br. J. Nutr.*, 23, 835–845 (1969).
- ³⁸ Sukhatme, P. V., *J. R. statist. Soc. A*, part 2, 166–000 (1974).
- ³⁹ Berkson, J., and Boothby, W. M., *Am. J. Physiol.*, 121, 669–683 (1938).
- ⁴⁰ Rutishauser, I. H. E., and Whitehead, R. G., *Br. J. Nutr.*, 28, 145–152 (1972).
- ⁴¹ Payne, P. R., *Am. J. clin. Nutr.*, 28, 218–286 (1975).
- ⁴² Beaton, G. H., and Swiss, L. D., *Am. J. clin. Nutr.*, 27, 485–504 (1974).
- ⁴³ Begum, A., Radhakrishnan, A. N., and Pereira, S. M., *Am. J. clin. Nutr.*, 23, 1175–1183 (1970).
- ⁴⁴ Whitehead, R. G., in *Proteins in Human Nutrition* (edit. by Porter, J. W. G., and Rolls, B. A.), 103–117 (Academic, London and New York, 1973).
- ⁴⁵ Lechtig, A., et al., *Am. J. Dis. Child*, 129, 553–556 (1975).
- ⁴⁶ Ministry of Overseas Development, *British Aid and the Relief of Malnutrition* (Overseas Development Paper no. 2) (HMSO, London, 1975).
- ⁴⁷ National Research Council Recommended Dietary Allowances; revised 1948, Publication No. 129; revised 1963, Publication No. 1146; seventh revised edition 1968, Publication No. 1694; eighth revised edition 1974 (US National Academy of Science, Washington DC, 1974).
- ⁴⁸ Food and Agriculture Organization, FAO Nutritional Studies No. 15; FAO Nutritional Studies No. 16 (FAO, 1957).
- ⁴⁹ FAO/WHO, *Wld Hlth Org. Tech. Rep. Ser. No. 301* (WHO, Geneva 1965).
- ⁵⁰ Department of Health and Social Security, *Report on Public Health and Medical Subjects No. 120* (HMSO, London, 1969).

articles

Lunar temperature and global heat flux from laboratory electrical conductivity and lunar magnetometer data

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Three-layer monotonic electrical conductivity models for the lunar interior to a depth of 600 km are used in conjunction with laboratory measurements of the electrical conductivity of olivine and pyroxene to estimate a temperature-depth profile. The temperatures calculated for depths of 400–600 km are consistent with attenuation of the seismic shear wave. The temperature calculated at a depth of 100–250 km yields a heat flow that is in good agreement with the directly measured lunar heat flow. The temperature, however, is sufficiently close to melting that mascon anisostasy would not be maintained. Thus a better conductor is required at this depth.

THE internal temperature of the moon has been calculated using a set of monotonic conductivity three-layer models derived from Lunar Surface Magnetometer (LSM) data¹ together with laboratory determined conductivity-temperature relationships for two minerals, orthopyroxene (OPX)² and olivine (OL)³, thought to be representative of the interior of the Moon.

The three-layer model is characterised by a core, shell, and crust. The latter has a vanishingly small electrical conductivity. Although the shell and core conductivities can be specified over a broad range of depths, only the conductivities at R_1 (the radius of the core) and R_2 (the outer radius of the shell) are really relevant. This is due to a very strong bias in favour of the outer edge of a layer, because this is where the relevant frequencies tend to be most damped⁴. Resolution tests (Schubert, personal communication) confirm this. Thus the shell conductivity refers to a depth of ~ 150–250 km, depending on circumstances to be discussed here, whereas the core conductivity refers to a depth of ~ 450–600 km. Deeper conductivity cannot be calculated with any confidence from the data in this paper.

For the monotonic three-layer conductivity model, variations in R_1 and R_2 occur because of uncertainty in the estimate of lunar higher order magnetic excitation. The addition to the nominal dipole excitation is primarily from the induced quadrupole moment. The multipolarity arises from higher orders of the field associated with finite wavelengths in the solar wind. For quadrupole and higher order, the direction of the convected wave fronts in the solar wind must be found to determine the pseudoscattering angle, θ . (The term pseudo-

scattering angle is used to signify that, although the theory of lunar induction is formally similar to Mie scattering, in the case of perfect confinement of the induced field, no field can be radiated. In the restricted theory of lunar induction, however, displacement currents are ignored, and thus only a near field is present.)

This angle, which affects estimates of the higher order induction, is defined as the angle between the phase front vector of the incoming radiation and the lunar radius vector to the site of the LSM. An additional variation in the magnitude of higher order induction arises from uncertainty in the wave phase velocity, U_p . This is generally less than the convected speed of the solar wind. (See refs 5, 6 for the scattering geometry.)

Here we restrict the pseudoscattering angle to $\theta = 150^\circ$, a reasonable approximation for a combination of Alfvén waves propagating down the spiral field direction and tangential discontinuities being convected in the solar wind. Changing either U_p or θ affects R_1 , R_2 and the respective conductivities similarly; therefore, we have chosen to vary U_p and maintain θ constant. In general, changes in θ do not produce major changes in the conclusions, except perhaps in the calculation of the heat flux.

Inversion of lunar transfer functions to yield a suite of monotonic three-layer models is based on spherically symmetric plasma confinement theory (SSP)⁷. This leads to an underestimate of electrical conductivity, because, on the Moon, field lines are carried from the front side to the diamagnetic cavity on the antisolar hemisphere, depressing the sunward response^{8,9}. This effect is expected to result in a slight upward revision of the temperature estimate when the full asymmetric theory, incorporating the angular spectrum of the wave field, is available.

The dependence of electrical conductivity on temperature was determined in the laboratory using mixed gases to maintain oxygen fugacity at a value within the stability field of the mineral to be measured. The electrical conductivity of the olivine used here is that reported by Duba *et al.*³ from the Red Sea. Conductivity has been determined with a gas mixture which produces an oxygen fugacity of 10^{-3} Pa (10^{-8} bar) at 1,200 °C. More experimental details and information on gas mixtures may be found in Duba and Nicholls¹⁰. The OPX conductivity was determined up to 1,400 °C on a sample of the Bamle enstatite² previously used for shock compression measurements¹¹. OPX₁ refers to measurements for $10^{-3} \leq f_{O_2} \leq 10$ Pa at 1,200 °C and hydrostatic pressure of 0.5 GPa; OPX₂ refers to measurements for $f_{O_2} = 10^{-3}$ Pa at

1,200 °C and hydrostatic pressure of 0.1 MPa. The electrical conductivity of the enstatite fits the formula

$$\sigma = \sigma_0 \exp(-\varepsilon/kT).$$

A change in the conduction mechanism at $\sim 1,300$ °C in the Bamle OPX is suggested by a change in σ_0 from ~ 10 to 30 MS m^{-1} and the activation energy, ε , from 1 to 3.2 eV.

Deep conductivity

Figure 1 shows the temperature at a depth between 450 and 600 km using OPX₁ (lower trapezoid), and OPX₂ and OL (upper trapezoid). In the latter case the temperatures derived from the two minerals are indistinguishable. Both the vertical and horizontal spreads are associated with the influence of higher order modes in the lunar excitation, which affects the calculation of temperature. The variations are due to changes in the assumed value of U_p where $200 \leq U_p \leq 400 \text{ km s}^{-1}$, changing the radii within the range $1,200 \leq R_2 \leq 1,280 \text{ km}$ and the electrical conductivity between $1.6 \times 10^{-3} \leq \sigma \leq 1.8 \times 10^{-3} \text{ S m}^{-1}$. The lower trapezoid shows a temperature range of only about 30 °C. All temperatures for R_1 are within ~ 100 °C, or less, of the Ringwood-Essene solidus¹². This close approach to the solidus is entirely consistent with the attenuation of seismic shear wave depths variously estimated to lie between 500 and 800 km (ref. 13). From calculations of Schubert *et al.*¹⁴ using a lunar model with an isothermal Fe core, convecting shell, and conducting lithosphere, the temperature at the depth discussed here is also consistent with those shown in Fig. 1 based on electrical conductivity.

Corrections to the data for diamagnetism and asymmetric induction would have opposite effects, the former to lower the estimated temperature and the latter to raise it. Diamagnetism requires a pressure gradient which balances the Lorentz force¹⁵. The slowing of the bulk flow of the solar wind ions at the Apollo 12 site has been attributed to interaction with the remanent field¹⁶. The slowing of the flow corresponds to the loss of $\sim 19\%$ of the free stream flow energy. This effect is the only report from lunar data from which evidence of a pressure gradient might be inferred. The effect of asymmetric induction is of the same order at high frequencies¹⁷.

Although a final assessment of the two effects cannot be made at present, the close correlation of Apollo 12 and 15 data suggests, in any event, that diamagnetism is not important. It could have a role only at Apollo 12, since the interaction with the solar wind is weak or nonexistent at Apollo 15.

For completeness, we have also included data in Fig. 1 from Dyal *et al.*¹⁸. The temperature extremes are calculated from their conductivity estimates using OPX₁. Their 'mean' is shown as the dashed line approximately bisecting their extremes, shown by the shaded region. The temperature profile from the present work is generally consistent with their 'mean', although their estimate of the near-surface temperature is sufficiently low to perhaps satisfy a single mineral composition Moon without involving the problem of mascon anisotasy discussed in the next section. Their deep temperature is depressed somewhat from our estimate, but sufficiently near the solidus to still infer the possibility of shear wave attenuation. The most recent analysis of transients by Dyal *et al.*¹⁹ shows a temperature profile, however, which exceeds the Ringwood-Essene solidus in the outer 500 km of the Moon.

Shell temperature and mascon anisotasy

The three-layer model gives two internal temperature estimates for a specified U_p . Varying U_p between the limits $200 \leq U_p \leq 400 \text{ km s}^{-1}$ and fixing $\theta = 150^\circ$ as before, the outer edge of the shell is $1,520 \leq R_2 \leq 1,600 \text{ km}$. The corresponding electrical conductivity is $2.9 \times 10^{-4} \leq \sigma \leq 5.5 \times 10^{-4} \text{ S m}^{-1}$ and the temperature is $1,110 \leq T_2 \leq 1,210$ °C. These values define the two trapezoids to the left side of Fig. 1. The conductivity at depth R_2 is determined strongly by the higher frequencies in

the solar wind. Thus higher order modes are here more important than for the conductivity at R_1 . For example, for $\theta = 180^\circ$ and $U_p = 200 \text{ km s}^{-1}$, $R_2 = 1,660 \text{ km}$ —a depth below the surface of only 80 km. We believe that real resolution at this depth is not attainable without higher frequency data. It is interesting to note that as the position of R_2 moves out with change in induction geometry, the electrical conductivity estimate is depressed, thereby tending to hold the thermal gradient fixed.

From Fig. 1 it is evident that the temperature calculated from OPX₁ is within 100 °C of the solidus of the model lunar pyroxenite of ref. 12. This proximity places a severe constraint on the rigidity of the lithosphere required to maintain the anisotasy of the circular mare mascons²⁰. Although the thickness of the lithosphere is uncertain and depends on the viscosity and thermal gradient, a thickness of at least 200–300 km is commonly assumed. We note that, even if the temperature estimate were reduced by estimations based on a less extreme portion of the trapezoids which encompass all the temperature estimates, little would be gained with regard to the rigidity problem. The barred portions of the trapezoids are used to denote that the temperature estimates are potentially in error because of the mascon problem, and may require downward revision. As we shall emphasise later, this implies a compositional change at this depth, but in turn raises difficulties with the heat flux from the Moon.

Global heat flux

A preliminary estimate of global heat flux can be obtained from the previous calculation of temperature at depths of 150–200 km. We have found that this is consistent with the direct heat

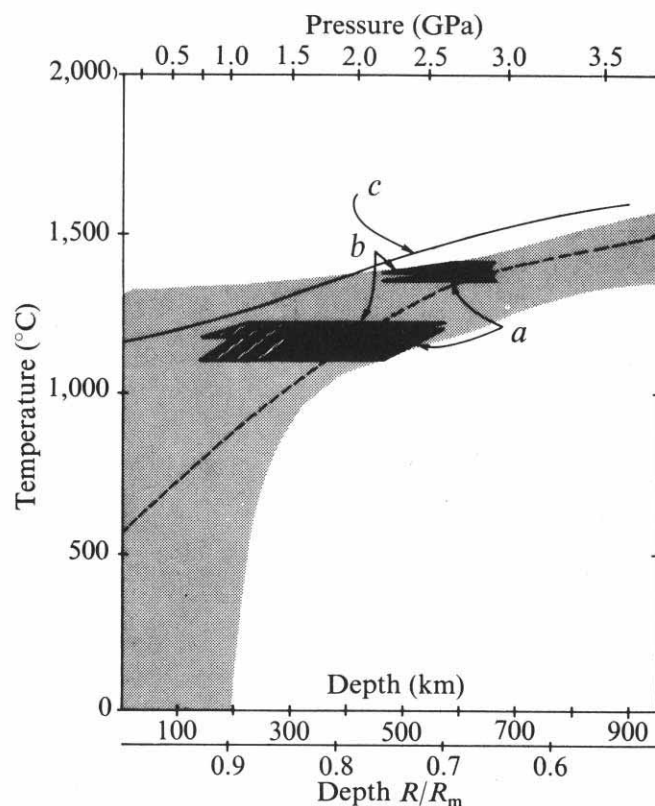


Fig. 1 Lunar temperature estimates from conductivity models *a*, orthopyroxene (OPX₁; ref. 2); $\sigma_0 = 3 \times 10^7 \text{ S m}^{-1}$, $\varepsilon = 3.32 \text{ eV}$ for $T > 1,327^\circ\text{C}$; $\sigma_0 = 2.03 \text{ S m}^{-1}$, $\varepsilon = 1.05 \text{ eV}$ for $T < 1,327^\circ\text{C}$. *b*, orthopyroxene (OPX₂; ref. 3); for $T > 1,327^\circ\text{C}$ the same as in *a*, $\sigma_0 = 3.49 \text{ S m}^{-1}$, $\varepsilon = 1.14 \text{ eV}$ for $T < 1,327^\circ\text{C}$; olivine³; $\sigma_0 = 17.4 \text{ S m}^{-1}$, $\varepsilon = 1.34 \text{ eV}$ for $T < 1,320^\circ\text{C}$. *c*, Solidus (ref. 12). Solid areas, obtained from three-layer monotonic model of Sonett *et al.*¹; shaded area, obtained from nightside and geomagnetic tail-lobe limits, based on OPX₁ (ref. 18); obtained from —, tail-lobe mean, based on OPX₁ (ref. 18).

Table 1 Thermal gradients calculated for varying U_p and $\theta = 150^\circ\text{C}$ in the three-layer monotonic conductivity model

U_p (km s ⁻¹)	R_2 (km)	T_{OPX_1} (°C)	T_{OPX_2} (°C)	T_{OL} (°C)	dT/dR ($10^6 \times ^\circ\text{C km}^{-1}$)		
					OPX ₁	OPX ₂	OL
400	1,519	1,210	1,240	1,230	5.6	5.7	5.7
300	1,537	1,190	1,220	1,210	6.0	6.0	6.1
200	1,600	1,140	1,140	1,140	8.2	8.4	8.4

flux measurements of Langseth *et al.*²¹ but is possibly too high, for reasons given in the previous section. Table 1 shows thermal gradients calculated assuming a linear increase of temperature with depth between the surface and R_2 . The thermal gradients are insensitive to variations in the relative amounts of OPX and OL. Thermal gradients (dT/dR) from Table 1 are used to construct Table 2, which shows estimates of heat flux, incorporating the variable U_p . To calculate heat fluxes in Table 2 we use an average thermal conductivity of $4.18 \times 10^{-2} \text{ W cm}^{-2} ^\circ\text{C}^{-1}$, a reasonable mean between a Hortonville dunite ($3.64 \times 10^{-2} \text{ W cm}^{-2} ^\circ\text{C}^{-1}$) and a Bushveldt pyroxene ($4.93 \times 10^{-2} \text{ W cm}^{-2} ^\circ\text{C}^{-1}$)²². Langseth *et al.*²¹ report a range of heat fluxes from 2.99–3.59 $\mu\text{W cm}^{-2}$ at the Hadley–Appenine site, with their best value being 3.31 $\mu\text{W cm}^{-2}$. Assuming that the most likely value for phase velocity is $200 \leq U_p \leq 300 \text{ km s}^{-1}$, as seems to be the most reasonable from arguments based on the solar wind data and studies of the lunar response, the magnetometer derived heat flux is 2.5–3.5 $\mu\text{W cm}^{-2}$. This is a global or large area average and is not dependent on local variations, but is model dependent, varying with U_p and compositional assumptions.

Discussion

Subsidence of mascons may have taken place over a long period of time, but this is uncertain; they are now below the mean surface of the Moon. There is some evidence that at least some shallow zone moonquakes are connected with ongoing subsidence. Lunar transient events are thought to correlate with the ringed maria and with shallow zone quakes²³. The Apollo 15 magnetometer anisotropy may require a dynamic process, suggesting a model of circumferential cracking at great depth. In spite of these indications of settling, the mascons seem to be anisostatic, and thus some crustal rigidity is required.

Actually the issue of mascons and isostasy seems unsettled because of the combination of lateral positive and negative anomalies across Serenitatis, for example. Hulme²⁴ produced a model with mass excess overlying mass deficiency to account for the negative gravity anomaly at the edges, but his model is only qualitatively correct and transforms the global problem into a problem of the stability of a more local region. He finds a negative anomaly only one-fourth of that which he quotes for Muller and Sjogren²⁵, and he states that it is difficult to provide quantitative consistency without changing his (Hulme's) model appreciably. Other models for the negative anomaly at the mare edge suggest a mass excess surrounded by a mass-deficient ring, correspondingly to crater excavation followed by filling^{26,27}. Current mascon models are non-unique, but in the light of this paper, the seismic data, and heat flow data, we think it more likely that the mascons exhibit large scale anisostasy. A ring of mass-deficient material is consistent with this, though it should be pointed out that Hulme's model cannot yet be conclusively ruled out.

If a rigid lithosphere is required on the Moon, then the temperatures estimated from the LSM and the electrical conductivity data on OPX and OL seem to be high, and the presence of a material having a higher electrical conductivity at a given temperature is required in the outer $200 \pm 50 \text{ km}$ of the Moon. We think that the problem of the source of the mare basalt is, as yet, too poorly understood to be able to infer a composition. The situation with the electrical conductivity–temperature functions for basalts is equally poorly understood.

Nevertheless it is worth noting that the electrical conductivity of terrestrial basalts, summarised by Duda and Lilley²⁸,

indicates that a lower temperature at a depth of $\sim 250 \text{ km}$ may result if the outer shell of the Moon were composed of basalt. The inferred depression of the temperature would strongly affect the estimates of dT/dR and the heat flux (H) shown in Tables 1 and 2. It should be noted that all electrical conductivity measurements of basalts have been made under conditions potentially favourable to oxidation of Fe^{2+} to Fe^{3+} . Since Duda and Nicholls¹⁰ have shown that the conductivity of olivine with Fe^{3+} may be increased by as much as three orders of magnitude, investigations of the electrical conductivity of basalt in conditions of controlled oxygen fugacity are now under way.

Table 2 Global heat flux $\mu\text{W cm}^{-2}$ estimated from Table 1

U_p (km s ⁻¹)	H_{OPX_1}	H_{OPX_2}	H_{OL}
400	2.35	2.40	2.38
300	2.51	2.57	2.56
200	3.41	3.51	3.52

Because of the difficulties with compositional models and electrical conductivity functions, we can only say that a substantial depression of the temperature at R_2 is required to satisfy the mascon-viscosity requirement. A guess is that the outer shell temperature at R_2 is between 600 and 1,200 °C, with prejudice towards the lower value. This would immediately depress the estimated global heat flux by about a factor of two. Since Langseth *et al.*²¹ inferred a global radionuclide concentration of about twice the chondritic value, the arguments given here would make the two approximately equal. These comments are based on the assumption that the Moon is at present in thermal equilibrium, a condition achievable in the present epoch only if strong convection has taken place.

Nakamura *et al.*¹³ infer a thermal gradient of $2 \leq dT/dR \leq 5^\circ\text{C km}^{-1}$ at a depth of 60 km (the bottom of their zone 1). This is a small thermal gradient, but their upper limit is consistent with a lowered global heat flux. It should be noted that the determination of the thermal gradient for the several experiments discussed here is for widely differing depths. We have assumed here that dT/dR is constant from the surface to R_2 , whereas the thermal gradient probably diminishes with depth. Thus Table 2 provides upper bounds for dT/dR . The heat-flow experiment measures the heat flux in the regolith, and therefore requires a secondary experiment to determine the thermal conductivity of the regolith, which cannot be compared directly with that at 150–250 km. It is likely that the radionuclide concentration is non-uniform and that the region near R_2 contains a significant fraction of the lunar radionuclide concentration. The outward migration of the radionuclide concentration inferred by these comments means that dT/dR would be increased in the layer very near to the surface, but possibly decreased near R_2 . Thus the question of the correct thermal gradient, and to which depth it refers is incompletely explored.

Conclusions

If the Moon is assumed to have a composition of OPX, OL, or a mixture of the two at a depth of 450–600 km, the temperature calculated from magnetometer data is near to the solidus of Ringwood and Essene¹². This high temperature is in accord with the evidence from seismic data of the attenuation of shear waves at a depth of 500–800 km. A temperature from nearer to the surface, at a depth of 150–250 km, is found by assuming

that the composition at shallow depth is similar to that assumed for 450–600 km. There the temperature is even closer to the melting curve. The thermal gradient inferred from the shallow temperature is consistent with the values found by Langseth *et al.*²¹, but cannot provide the lithospheric rigidity needed for support of the mascons. A reduction in the estimate of lithospheric temperature can be made only if a more conducting material is present at the depths of about 150–250 km. At the same time this reduction in the lithospheric temperature depresses the calculated heat flux. Depending on the material composition, the temperature estimate using the results of Langseth *et al.*²¹ could be reduced by a factor of nearly two. If heat-producing radionuclides were concentrated in the outer 60 km, the thermal gradient derived in this paper would be more in accord with that estimated by Nakamura *et al.*¹³. The reconciliation of dT/dR at 60 km inferred from the seismic data and that inferred from the conductivity data, requires further study to find the curvature in the thermal profile, that is d^2T/dR^2 .

Substances which are consistent with geophysical constraints for the lithosphere, derived from the magnetometer data, include possibly basalts, but this may be inconsistent with geochemical evidence. Recent electrical conductivity measurements on the albite end member of the plagioclase series indicate that plagioclase in the lunar upper mantle would yield lower temperatures than olivine and pyroxene²⁹. Petrologic models which envision an ilmenite-rich layer³⁰ in this region would conceivably also allow higher electrical conductivity at lower temperatures. The available magnetometer data cannot provide details of the conductivity profile; but the requirement that the conductivity–temperature dependence be greater, that is, higher conductivity for the same temperature, could easily mean a non-monotonic conductivity profile. It is unlikely, noting the requirement for a change of composition to more conducting material, that the conductivity–depth relation is monotonic. Thus the alternate model of the interior conductivity, with a narrow region of high conductivity, remains electrically valid³¹ and is a plausible petrological and geophysical model.

Finally we note that in the region between R_1 and R_2 , the thermal gradient, $dT/dR \sim 1^\circ\text{C km}^{-1}$, whereas the adiabatic lapse rate at these depths is $\sim 10^{-2}^\circ\text{C km}^{-1}$. It is unsafe to speculate on the possibility of solid state convection at this depth because of the paucity of data, but the evidence is not favourable. Convection at greater depths is still a possibility.

It should be noted that uncertainty about the lithospheric composition would even apply if there were a little water present. Although there seem to be compelling geochemical arguments against this, it would be unsafe to draw an absolute conclusion regarding H_2O . The effect of water on the electrical conductivity has not been fully investigated.

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- ¹ Sonett, C. P., Smith, B. F., Colburn, D. S., Schubert, G., and Schwartz, K., *Proc. third Lunar Sci. Conf., Geochim. Cosmochim. Acta, Suppl.* 3, 3, 2309 (1972).
- ² Heard, H. C., Duba, A., Piwinski, A. J., and Schock, R. N., in *Lunar Sci. VI, Abstracts of papers presented at the Sixth Lunar Sci. Conf., Houston, Part I*, 355 (1975).
- ³ Duba, A., Heard, H. C., and Schock, R. N., *J. geophys. Res.*, 79, 1667 (1974).
- ⁴ Sonett, C. P., Schubert, G., Smith, B. F., Schwartz, K., and Colburn, D. S., *Proc. second Lunar Sci. Conf., Geochim. Cosmochim. Acta*, 3, 2415 (1971).
- ⁵ Schubert, G., and Schwartz, K., *J. geophys. Res.*, 77, 76 (1972).
- ⁶ Sonett, C. P., and Colburn, D. S., in *Planets, Stars and Nebulae Studied with Photopolarimetry* (edit. by Gehrels, T.), 419 (Univ. of Ariz. Press, Tucson, 1974).
- ⁷ Schubert, G., and Schwartz, K., *The Moon*, 1, 106 (1969).
- ⁸ Schubert, G., Sonett, C. P., Schwartz, K., and Lee, H. J., *J. geophys. Res.*, 78, 2094 (1973).
- ⁹ Smith, B. F., Colburn, D. S., Schubert, G., Schwartz, K., and Sonett, C. P., *J. geophys. Res.*, 78, 5437 (1973).
- ¹⁰ Duba, A., and Nicholls, I. A., *Earth Planet., Sci. Lett.*, 18, 59 (1973).
- ¹¹ Ahrens, T. J., and Gaffney, E. S., *J. geophys. Res.*, 76, 5504 (1971).
- ¹² Ringwood, A. E., and Essene, E., *Proc. Apollo 11 Lunar Sci. Conf., I, Geochim. Cosmochim. Acta, Suppl.* 1, 769 (1970).
- ¹³ Nakamura, Y., *et al.*, *Geophys. Res. Lett.*, 1, 137 (1974).
- ¹⁴ Schubert, G., Young, R. E., and Cassen, P., *Phil. Trans. R. Soc.* (in the press).
- ¹⁵ Spitzer, L., *Physics of Fully Ionized Gases* (Interscience, New York, 1956).
- ¹⁶ Neugebauer, M., Snyder, C. W., Clay, D. R., and Goldstein, B. E., *Planet. Space Sci.*, 20, 1577, (1972).
- ¹⁷ Schubert, G., Smith, B. F., Sonett, C. P., Schwartz, K., and Colburn, D. S., in *Lunar Sci. VI, Part II*, 720, 1975.
- ¹⁸ Dyal, P., Parkin, C. W., and Daily, W. D., *Proc. Fifth Lunar Sci. Conf., Geochim. Cosmochim. Acta, Suppl.* 5, 3, 3059 (1975).
- ¹⁹ Dyal, P., Parkin, C. W., and Daily, W. D., in *Lunar Sci. VI, Abstracts of papers presented at the Sixth Lunar Sci. Conf., Houston, Part I*, 226 (1975).
- ²⁰ Arkani-Hamed, J., *The Moon*, 6, 100 (1973). Arkani-Hamed, J., *Proc. Fifth Lunar Sci. Conf. Geochim. Cosmochim. Acta, Suppl.* 5, 3, 3127 (1974).
- ²¹ Langseth, M. G., Jr, Clark, S. P., Jr, Chute, J. L., Jr, Keihm, S. J., and Wechsler, A. E., *The Moon*, 4, 390 (1972).
- ²² *Handbook of Physical Constants* (edit. by Clark, S. P., Jr) (Geological Society of America, Washington DC, 1966).
- ²³ Runcorn, S. K., *Proc. Fifth Lunar Sci. Conf., Geochim. Cosmochim. Acta, Suppl.* 5, 3, 3115 (1974).
- ²⁴ Hulme, G., *Nature*, 238, 448 (1972).
- ²⁵ Muller, P. M., and Sjogren, W. L., *Science*, 161, 680 (1968).
- ²⁶ Phillips, R. J., *J. geophys. Res.*, 79, 2027, (1974).
- ²⁷ Phillips, R. J., Conel, J. E., Abbott, E. A., Sjogren, W. L., and Morton, J. B., *J. geophys. Res.*, 77, 7106 (1972).
- ²⁸ Duba, A., and Lilley, F. E. M., *J. geophys. Res.*, 77, 7100 (1972).
- ²⁹ Piwinski, A. J., and Duba, A., *Proc. Sixth Lunar Sci. Conf., Geochim. Cosmochim. Acta, Suppl.* 6, 1 (in the press).
- ³⁰ Kesson, S., *Proc. Sixth Lunar Sci. Conf., Geochim. Cosmochim. Acta, Suppl.* 6, 1 (in the press).
- ³¹ Sonett, C. P., *et al.*, *Nature*, 230, 359 (1971).

Cytological detection of mutagen–carcinogen exposure by sister chromatid exchange

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A staining technique that detects sister chromatid exchanges (SCEs) has been used to examine the response of chromosomes in cultured Chinese hamster cells to a wide variety of mutagens–carcinogens. The test gives a very sensitive and rapid method for detecting chromosome mutagenicity of chemical agents and provides a powerful new method for detecting environmental mutagens.

SISTER chromatid exchange (SCE) involves a symmetrical exchange at one locus between sister chromatids, which does not result in an alteration of overall chromosome

morphology. Such exchanges were originally demonstrated by Taylor¹ in autoradiographic studies of plant chromosomes and were seen as switches in radioactive label between chromatids at the second (M_2) mitosis after ^3H -thymidine labelling at the S phase of the M_1 cycle. Later studies showed that many of the SCEs observed in autoradiographic experiments were induced by the endogenous radiation from the incorporated tritium²; that the frequency of SCE was increased in cells exposed to X rays³, ultraviolet light^{4,5} and certain chemical mutagens⁶; and that a significant proportion of gross chromatid aberrations (including deletions) seemed to involve an SCE event at the site of aberration⁷.

Techniques have been developed which distinguish between sister chromatids without using radioisotopes and autoradiography. These involve exposing cells to 5-bromo-deoxyuridine (BUdr) for two rounds of replication so that M_2 chromosomes possess one chromatid unifilarly substituted with BUdr and its sister bifilarly substituted. Such chromatids stain differentially with Giemsa⁸, the fluorochrome Hoechst 33258⁹ (and various other fluorescent dyes, for example, acridine orange and quinacrine), or a combination of fluorochrome plus Giemsa—the FPG technique of Perry and Wolff¹⁰. At least a proportion of SCEs observed in BUdr-treated cells are induced by the analogue¹¹, but Latt¹² has demonstrated a fourfold increase in SCE in BUdr-treated human lymphocytes exposed to the chemical mutagen mitomycin C (MMC) at a dose which causes only a low frequency (0.07 per cell) of chromatid aberrations.

We have confirmed Latt's¹² finding and, since the FPG technique provides an easy, rapid and high resolution method for detecting SCE, investigated whether the induction of SCE could provide a simple and sensitive endpoint for assaying mutagenicity in mammalian cells. We now present some of our results obtained with a line of Chinese hamster fibroblasts (CHO) exposed *in vitro* to fifteen mutagens—carcinogens, each in a range of concentrations (doses). Our findings from these experiments, and from preliminary studies on patients treated with cytotoxic drugs, strongly suggest that the SCE test provides a powerful tool for *in vitro* mutagen testing and possibly for monitoring people exposed to known or suspected mutagens—carcinogens.

In all the *in vitro* experiments CHO cells were used and cultured as described previously¹¹. Exponentially growing cells were exposed to 10 μ M BUdr for two cycles of DNA replication before treatment with colcemid (2 h at 2×10^{-7} M), collection by mitotic shake-off¹³, pretreatment for 7 min with 0.075 M KCl and fixation in 3:1 methanol-acetic acid. Throughout, all cultures were maintained in the dark or under a yellow safe light to minimise photolysis of BUdr and background SCE⁸. Air-dried preparations were made, stained by the FPG procedure¹⁰ and coded and scored for SCE and chromosomal aberrations by one observer.

X rays

SCE were scored in "harlequin-stained" (Fig. 1) chromosomes at the first mitosis after acute (50 rad min⁻¹) X-ray exposures at various doses. Using synchronously developing cultures (see legend for Fig. 2), the responses of cells exposed in G_1 , S and G_2 were determined (Fig. 2). The incorporation of BUdr into DNA enhances its sensitivity to damage by both X rays¹⁴ and ultraviolet light¹⁵. In our experiments chromatids in G_1 cells were all unifilarly substituted with BUdr at the time of irradiation, whereas half of the G_2 chromatids were bifilarly substituted. Our data demonstrate, however, that the chromosomes in G_1 cells showed no increase in SCE over background at any of the doses, whereas there were significant dose-dependent increases in S and G_2 cells.

Studies on chromatid aberrations induced by certain chemical mutagens^{16,17} have shown that cells exposed in G_2 do not yield aberrations at the ensuing mitosis, but do so at the subsequent division, the development of aberrations necessitating an intervening DNA replication between treatment and observation. Somewhat similar, but not identical, findings have also been reported with ultraviolet light^{18,19}. With ionising radiations, however, irradiation of G_2 cells results in the formation of aberrations which are seen at the first mitosis after exposure, and although there is some evidence for a small increase in aberrations in irradiated G_2 cells at their second mitosis¹⁹, it is clear that a large proportion of the lesions that develop into aberrations do so

shortly after their formation without the need to pass through a replication phase. Our data on the X-ray induction of SCE, however, closely parallel those obtained for aberrations with ultraviolet light and many chemical mutagens, suggesting that the X-ray-induced lesions resulting in SCE are relatively long lived (and are presumably therefore not single strand breaks²⁰), become manifest as SCE events as a consequence of DNA replication and reflect a type of recombinational repair process.



Fig. 1 M_2 metaphase cell from a Chinese hamster cell line (CHO) after two rounds of replication in the presence of BUdr (10 μ M) and stained with the FPG technique. Note the differentiation between chromatids and the presence of 12 SCEs.

In the context of this report, we emphasise that although there is a linear or curvilinear relationship between X-ray dose and SCE yield in G_1 and S cells, the increased incidence of SCE is minimal in relation to the induced incidence of chromosome and chromatid aberrations and this contrasts markedly with our results on chemical mutagens (see below). For example, at an X-ray dose which doubles the SCE incidence (~400 rad) there is a more than twentyfold increase in aberration frequency. This would imply that the induction of X rays of the kinds of lesions that result in SCE is inefficient relative to those events that result in aberrations, or, that most of these lesions are repaired (and a proportion gives rise to aberrations?) outside the S phase. In any case it seems clear that counting the incidence of SCE does not seem to provide a sensitive system for detecting X-ray exposure.

Chemical agents

The effectiveness of a chemical as a mutagen depends on various factors. Some mutagens are highly reactive with a short half life *in vivo* and may be very unstable in solution so that treatment with such agents, for example, sulphur mustard and to a lesser extent methyl and ethyl methane sulphonates (MMS and EMS) and N-methyl-N-nitro-N-nitrosoguanidine (MNNG), is essentially in the form of an acute or semi-acute exposure. Other mutagens, for example, mitomycin C (MMC), are much more stable and their continued presence effectively results in a chronic exposure. The activity of most agents is very dependent on pH and

some, for example, cyclophosphamide, are not active *per se*, but are metabolised within the body to give mutagenic metabolites. Much human exposure to environmental chemical mutagens may be considered to be akin to a chronic exposure, so that in the present experiments all chemical agents were introduced into the culture medium at the time of addition of BUdr and the medium was not changed until the cells were sampled 24–36 h later.

Most of the agents selected for study were chemically unrelated, but all were proven (or suspected) mutagens or carcinogens. Many caused considerable delays in cell progression and these delays were assessed for each concentration of chemical in preliminary experiments in which mitotic cells were collected at various intervals after 24 h, and final sample times were then selected to give good yields of M_2 cells. Virtually all control cells sampled after 24 h were in the M_2 division, whereas with sub-lethal doses of many agents, samples collected as late as 36 h contained a high proportion of M_1 cells. These delayed M_1 cells showed a very high frequency of chromosomal aberrations compared with M_2 cells on the same slides (Table 1). It is important to realise therefore that cells scored for SCE at high concentrations represent selected samples which must give an underestimate of the maximum SCE frequency.

The chemical agents studied, and the relationship between initial dose (concentration) and SCE frequency in M_2 cells, are shown in Fig. 3. The alkylating agents, and in particular the bifunctional compounds—MMC and nitrogen mustard (HN_2 , Fig. 4)—are extremely potent inducers of SCE in CHO cells, with MMC giving a doubling of SCE frequency at 10^{-8} M. The monofunctional alkylating agents, on the other hand, are generally less effective and show a considerable range in their ability to induce SCEs. This result is consistent with the finding that monofunctional agents are usually less efficient at inducing chromosomal aberrations than their bifunctional analogues²¹. Monofunctional compounds, however, may be more efficient in inducing mutations in bacteria and bacteriophage²¹. It is also notable that of the sulphonates, MMS is a more potent inducer of SCE than EMS, and this is also true for the induction of chromosomal aberrations. Thus, the ability of an alkylating agent to induce SCEs seems to bear no simple direct relationship to its efficiency in inducing point mutations in bacteria, but is more closely related to its ability to induce chromosomal aberrations in eukaryotes.

Quinacrine mustard (QM) produces chromosomal aberrations and is a monofunctional alkylating agent which may also intercalate between the bases in DNA²². Other intercalating agents produce frame shift mutations in bacteria as a consequence of distorting the double helix, and QM and the two other intercalating agents we studied, the anti-tumour²³ antibiotic adriamycin and the fluorochrome Hoechst 33258, a dibenzimidazol derivative²⁴, are also very effective inducers of SCE, with adriamycin being a very efficient inducer of chromosomal aberrations²⁵. Chromosomal aberrations are also induced by the potent carcinogen²⁶ and mutagen²⁷ 4-nitro-quinoline 1-oxide (4NQO), which is also a very efficient inducer of SCE.

Figure 3 shows that two of the fourteen chemical agents studied induced no SCE. Maleic hydrazide (MH), which is widely used as a herbicide, is a potent inducer of chromo-

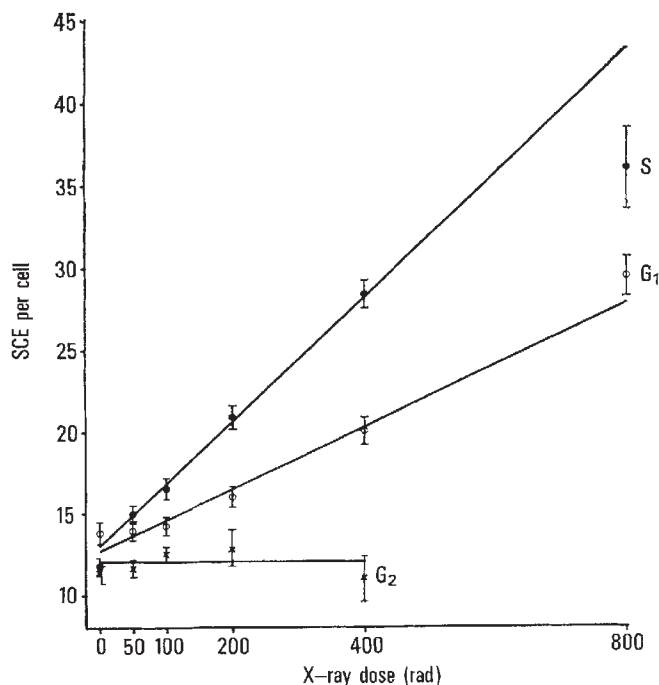


Fig. 2 The relationship between X-ray dose and SCE frequency in M_2 cells exposed to radiation in G_1 (○), S (●) and G_2 (×) of the second cell cycle. All irradiations were carried out on cells which had completed at least one round of replication in BUdr, using a Siemens Stabilipan X-ray set giving 50 rad min⁻¹ at 250 kV and 15 mA after filtration with 0.8 mm Su, 0.25 mm Cu and 1 mm Al. Cells exposed in G_2 were treated with colcemid 90 min after exposure of asynchronously developing populations and collected by mitotic shake-off 2.5 h later. For G_1 and S exposures, cells were synchronised by mitotic shake-off after one cell cycle of culture in the presence of BUdr (11–12 h). Serial shake-offs were performed every 15 min and cell samples with a mitotic index $\geq 98\%$ were collected and pooled, on ice, to give sufficient cells for replating at 37 °C and irradiation. Cells were irradiated 90 min (G_1) or 7.5 h (S) after plating and cell stages were checked by autoradiography of parallel ³H-thymidine labelled cultures. G_1 and S cells were maintained in BUdr medium and exposed to colcemid before collection 17 h and 13 h after irradiation. Sampling times were adjusted to take account of mitotic delay and SCEs were scored in metaphase cells with harlequin-stained chromosomes characteristic of cells completing two rounds of replication in the presence of BUdr (Fig. 1). The lines in the figure were drawn by eye and each point represents a mean $\pm$ s.e. from 50 cells.

somal aberrations in plant cells when applied in solution at low pH²⁸. It has never been reported to cause chromosome damage in mammalian cells and in our experiments, where pH was maintained at 7.2 using HEPES buffer, it induced neither aberrations nor SCE. The other compound which had little effect on SCE was the antineoplastic²⁹ agent cyclophosphamide. In itself, cyclophosphamide is ineffective as a cytostatic or chromosome damaging drug, but it is metabolised in the liver to give a very active bifunctional alkylating intermediate³⁰; its inefficiency in producing aberrations and SCE in our *in vitro* system is therefore hardly surprising.

Table 1 Frequencies of chromosome aberrations in 34-h samples of cells exposed to 10^{-7} M mitomycin C (50 cells per sample)

Treatment	Chromosome aberrations				Chromatid aberrations		
	Dicentric	Rings	Deletions	Gaps/Breaks	Rings	Isochromatid	Interchanges
MMC only	1	1	—	1	—	4	5
MMC+BUdr	—	6	—	—	2	—	4
M_2 cells only	—	6	—	—	2	—	4
MMC+BUdr	—	6	—	—	2	—	4
M_1 cells only	1	4	1	7	1	2	9

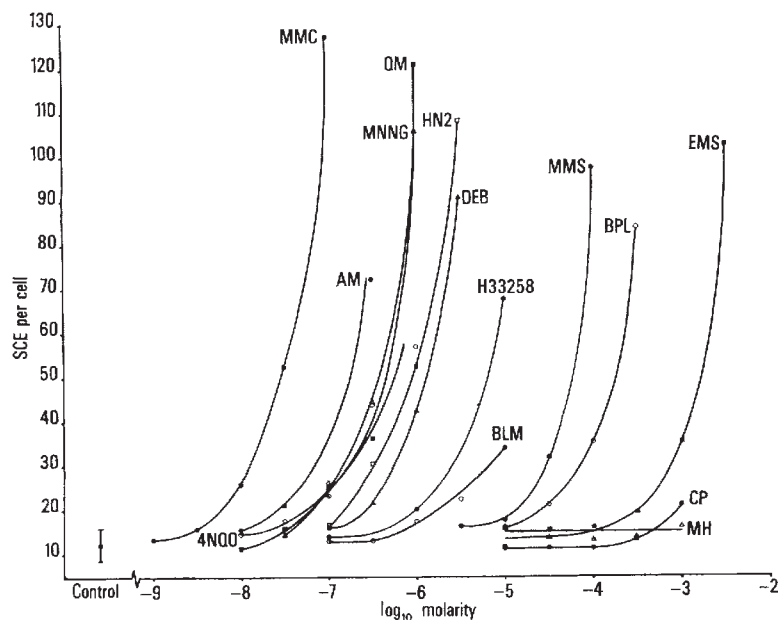


Fig. 3 Dose-response curves for SCE incidence in CHO cells against log of initial concentration of chemical agent, for the fourteen chemical agents studied. All chemicals were added to culture medium at the time of addition of BUdr and the medium was not changed until collection (see text). Those agents that were insoluble in water were first dissolved in a small volume of ethanol before dilution in tissue culture medium and all dispensing was completed in 8 min. Collection times were adjusted to take account of mitotic delay and cultures were maintained at 37 °C. SCEs were scored in harlequin-stained chromosomes of second division cells, and each point on the graph represents a mean frequency of SCE from 20 cells (~400 chromosomes); standard errors are not shown since for all points they were less than 5% of the mean frequency. MMC, Mitomycin C; AM, adriamycin; MNNG, N-methyl-N-nitro-N-nitrosoguanidine; QM, quinacrine mustard; 4NQO, 4-nitroquinoline 1-oxide; HN2, nitrogen mustard; DEB, diepoxy-butane; H33258, Hoechst 33258; BLM, bleomycin; MMS, methylmethane sulphonate; BPL, β -propiolactone; EMS, ethyl methane sulphonate; CP, cyclophosphamide; MH, maleic hydrazide.

All the tested agents which induce SCE also induce chromosomal aberrations, and an important practical question is whether these agents produce significant increases in SCE at doses which induce only a negligible or low aberration frequency. We have examined this question by scoring for aberrations at doses which give a doubling of SCE incidence, and for doses which result in a tenfold increase in SCE. The results are summarised in Table 2. In considering these data it must of course be remembered that SCE can only be scored in M_2 cells and, as already indicated (Table 1), the frequency of chromosomal aberrations is often higher in M_1 cells. Some of the aberrations were induced by the BUdr itself³¹ (Table 1) and most of

the chemical agents were found to induce chromatid-type changes. Most of the chromosome-type aberrations in the M_2 cells listed in Table 2 were "derived"³² by duplication from chromatid-type aberrations produced in the first cell cycle. The data in Table 2 show that for all but one (bleomycin) of the twelve mutagens-carcinogens studied, a dose of mutagen that gives a highly significant doubling of the incidence of SCE produces only a minimal and, with the small number of cells scored, barely noticeable effect on the incidence of chromosomal aberrations. Doses giving tenfold increases in SCE gave increased, but still very low, aberration frequencies.

The scoring of chromosomal aberrations requires a high

Table 2 Total chromosomal aberration frequency and total SCE in 20 M_2 cells scored at each of two doses for each chemical agent

Chemical	Chromosome aberrations (B')				Chromatid aberrations (B')			Total B' + B'	Total SCE
	Dicentric	Rings	Deletions	Gaps/breaks	Rings	Isochromatid	Interchanges		
Control									
BUdr only	1	1		1				3	244
AM 3×10^{-8} M				1		1		2	430
3×10^{-7} M				++	++	++	++	++	1,444
EMS 3×10^{-4} M		2	2					4	382
3×10^{-3} M			1	1			1	3	2,054
MMS 3×10^{-5} M		1						1	644
3×10^{-4} M		1						1	1,952
MMC 10^{-8} M		2	3	1				6	512
10^{-7} M								0	2,555
HN ₂ 3×10^{-2} M								0	612
3×10^{-6} M		2				1		3	2,175
4NQO 10^{-7} M								0	462
10^{-6} M			1					1	1,140
MNNG 10^{-7} M		1	2					3	528
10^{-6} M		4		3	2		3	12	2,124
DEB 3×10^{-7} M			1			1		2	403
3×10^{-6} M		4						4	1,818
BLM 3×10^{-6} M				++	++	++	++	++	451
QM 10^{-7} M	—	—	—	—	—	—	—	—	—
10^{-6} M		1	4					5	2,422
CP 10^{-3} M		1	1					2	424
BPL 3×10^{-5} M		2						2	425
3×10^{-4} M		3	1				1	5	1,668

The two doses selected were those which resulted in an approximate doubling and an approximate tenfold increase in SCE. With all agents except bleomycin, a doubling dose for SCE resulted in no obvious increase in chromosomal aberrations in the M_2 cells scored, and, in most cases, a dose giving a tenfold increase in SCE gave only a very much smaller increase in chromosomal aberrations. With both bleomycin and adriamycin at 3×10^{-7} M, there were too many chromatid-type aberrations present for accurate scoring, and this is indicated by ++.

level of skill and is very time consuming, since even at fairly high doses of mutagen the analysis of up to 100 cells or so per dose, which may involve several hours at the microscope, may be necessary to obtain significant results. Our findings show that through determining SCE frequencies it is possible to detect significant effects of mutagens on chromosome structure at doses that are usually very much lower than those necessary to produce marked increases in chromosomal aberrations. Moreover, the scoring of SCEs requires little training and is extremely rapid, so that, for example, the detection of a doubling of background incidence, at the 5% level of significance, requires the scoring of no more than 10–20 cells, which takes a matter of a few minutes.

In vivo studies on man

We have shown³³ that the incidence of SCE in human blood lymphocytes is relatively constant between individuals, and is independent of age and sex. The notable exception here is the very high SCE incidence in cultured cells from patients with Bloom's syndrome³⁴, an increase which is not



Fig. 4 M2 CHO cell after exposure to BUdr and HN₂ (3×10^{-6} M) throughout the two cell cycles before sampling. Note the approximately tenfold increase in SCE relative to the example from controls in Fig. 1.

evident in cells from patients with other inherited chromosome instability syndromes^{33,34}. This stability, and the very high sensitivity of what we have referred to as the SCE test, has therefore led us to look for increases in SCE frequencies in peripheral blood lymphocyte chromosomes of patients undergoing therapy with cytotoxic drugs. Only preliminary results are available and our detailed findings will be presented elsewhere. But it is clear from our first results on patients treated with adriamycin that significant increases in SCE are to be observed in blood cells sampled 24 h after injection with this drug, at dose levels which produce only a negligible frequency of chromosomal aberrations in these cells. It seems possible therefore that we may be able to use SCE incidence to detect significant exposures of individuals to mutagens–carcinogens.

Implications and applications

Our results show that the marked increase in incidence of SCE in cells exposed to a wide variety of chemical

mutagens is associated with very much smaller increases in chromosome aberration yields, whereas the converse holds in cells exposed to X radiation. Chromosomal aberrations are of course mutational events, although many will be expressed as dominant lethals in germ cells. A very relevant question therefore is whether SCE events are themselves associated with mutational changes? This question is difficult to answer, but we believe that at least some sister chromatid exchanges may be associated with mutation. Our reasons for this belief are based on two arguments. First, SCE is often seen to have occurred at the site of chromatid breakage, or intrachange resulting in duplication and/or deletion. It is evident therefore that incomplete or abnormal SCE events do occur, and that these certainly result in aberrations and mutations. Second, the exchange event that results in SCE involves recombination between parent and daughter polynucleotide strands at sites of transient discontinuity in both parental strands¹¹. Our data suggest that this recombination repair process³⁸ takes place at the time of DNA replication and it seems not improbable, as has been demonstrated in bacteria³⁷ and meiotic cells of yeast³⁸, that mutation originates as an error in the recombination repair process following mispairing and base deletion or insertion.

Our data show that SCE frequencies are far more sensitive indices of chromosome damage than frequencies of gross chromosomal aberrations, and that the new techniques to detect them have provided a simple, reliable and highly sensitive method for assaying the chromosome mutagenicity of environmental agents. Only further work will establish whether these techniques will provide a good system for monitoring human exposure, but we suggest that the *in vitro* SCE test on human or other mammalian cells should form part of any environmental mutagen testing programme.

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- 1 Taylor, J. H., *Genetics*, **43**, 515–529 (1958).
- 2 Gibson, P. A., and Prescott, D. M., *Expl Cell Res.*, **74**, 397–402 (1972).
- 3 Brewen, J. G., and Peacock, W. J., *Mutat. Res.*, **7**, 433–440 (1969).
- 4 Marin, G., and Prescott, D. M., *J. Cell Biol.*, **21**, 159–167 (1964).
- 5 Gatti, M., and Olivieri, G., *Mutat. Res.*, **17**, 101–112 (1974).
- 6 Kato, H., *Expl Cell Res.*, **82**, 383–390 (1973).
- 7 Rommelare, J., Susskind, M., and Errera, M., *Chromosoma*, **41**, 243–247 (1973).
- 8 Kato, H., *Expl Cell Res.*, **85**, 239–247 (1974).
- 9 Heddle, J. A., and Bodycote, D. J., *Mutat. Res.*, **9**, 117–126 (1970).
- 10 Ikushima, T., and Wolff, S., *Expl Cell Res.*, **87**, 15–19 (1974).
- 11 Latt, S. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3395–3399 (1973).
- 12 Perry, P. E., and Wolff, S., *Nature*, **251**, 156–158 (1974).
- 13 Wolff, S., and Perry, P. E., *Chromosoma*, **48**, 341–353 (1974).
- 14 Latt, S. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3162–3166 (1974).
- 15 Terasima, T., and Tolmach, L. J., *Nature*, **190**, 1210–1211 (1961).
- 16 Dewey, W. C., and Humphrey, R. M., *Mutat. Res.*, **23**, 538–553 (1965).
- 17 Regan, J. D., Setlow, R. B., and Ley, R. D., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 708–712 (1971).
- 18 Evans, H. J., and Scott, D., *Genetics*, **49**, 17–38 (1964).
- 19 Evans, H. J., and Scott, D., *Proc. R. Soc. B*, **173**, 491–512 (1969).
- 20 Griggs, H. G., and Bender, M. A., *Science*, **179**, 86–88 (1973).
- 21 Ikushima, T., and Wolff, S., *Mutat. Res.*, **22**, 193–201 (1974).
- 22 Evans, H. J., *Genetics*, **79**, Suppl., 171–178 (1975).
- 23 Loveless, A., *Genetic and Allied Effects of Alkylating Agents* (Butterworth, London, 1966).
- 24 Modest, E. J., in *Twenty-third Nobel Foundation Symp. Chromosome Identification* (edit. by Caspersson, T., and Zech, L.), 323–326 (Academic, New York, 1973).
- 25 Bonadonna, G., Monfardini, S., De Lena, M., and Fossati-Bellani, F., *Br. med. J.*, **3**, 503–506 (1969).
- 26 Gropp, A., Hilwig, I., and Seth, P. K., in *Symposia Medica Hoechst*, **6**, 149–155 (Shattauer, Stuttgart, 1973).
- 27 Vig, B. K., *Cancer Res.*, **31**, 32–38 (1971).
- 28 Nakahara, W. F., Fukuoka, F., and Sakai, S., *Gann*, **49**, 33–41 (1958).
- 29 Stich, H. F., and San, R. H. C., *Mutat. Res.*, **10**, 389–404 (1970).
- 30 Kihlman, B. A., *J. biophys. Biochem. Cytol.*, **2**, 543–555 (1956).
- 31 Arnold, H., Bourseaux, F., and Brock, N., *Nature*, **181**, 931 (1958).
- 32 Connors, T. A., Cox, P. J., Farmer, R. B., Foster, A. B., and Jarman, M., *Biochem. Pharmacol.*, **23**, 115–129 (1974).
- 33 Hsu, T. C., and Somers, C. E., *Proc. natn. Acad. Sci. U.S.A.*, **47**, 396–403 (1961).
- 34 Evans, H. J., in *Human Population Cytogenetics*, Pfizer Medical Monographs, **5**, (edit. by Jacobs, P. A., Price, W. H., and Law, P.), 191–216 (Edinburgh University Press, Edinburgh, 1970).
- 35 Galloway, S. M., and Evans, H. J., *Cytogenet. Cell Genet.*, **15**, 17–29 (1975).
- 36 Chaganti, R. S. K., Schonberg, S., and German, J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4508–4512 (1974).
- 37 Sperling, K., Wegner, R. D., Richm, H., and Obe, G., *Humangenetik*, **27**, 227–230 (1975).
- 38 Howard-Flanders, P., *Br. med. Bull.*, **29**, 226–235 (1973).
- 39 Witkin, E. M., in *Ann. Rev. Genet.* (edit. by Roman, H. L.), **3**, 525–552 (Annual Reviews Inc., Palo Alto, 1969).
- 40 Magni, G. E., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 975–980 (1963).

Polarity of structure and of ordered nerve connections in the developing amphibian brain

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Tectal polarity for retinal connections remains reversible long after the anatomical pattern of neural structures has been determined. Cells in the diencephalon seem to control this polarity. Following certain embryonic operations, the diencephalon developed behind the tectum. In such cases, the polarity of the retino-tectal projection was reversed.

THE amphibian retino-tectal projection has been widely used for studying the mechanisms governing selective nerve connections. One important fact which has emerged from the study of this system is that critical events occurring early in ontogeny determine the axial polarity of the retina with respect to its connections to the tectum, the primary visual centre. When an eye in a stage 32 *Xenopus*¹ is rotated by 180°, cells in the original anterior pole of the retina connect to the posterior pole of the tectum, as they would have done if left undisturbed, and the animal's visual world is accordingly rotated by 180°. If a similar operation is performed some 7 h earlier (Stage 28 in *Xenopus*), the animal develops normal visuo-tectal connections²⁻⁵, because of repolarisation of the eye in the orbit.

It has been conjectured⁶ that an equivalent determination of axial polarity occurs within the tectum, to which retinal cells later connect through their optic nerve fibres. The time of tectal polarisation is not known, for the only attempted rotation experiments on the tectum comparable with those on the embryonic eye did not give clear results⁷. If such tectal polarisation does take place, it is likely that the information for this, whatever its nature, may originate from a local group of cells, as has been demonstrated for the control of spatial patterns of cellular differentiation early in embryonic development^{8,9}.

Here we report briefly on the results of an investigation directed at these questions. Our findings indicate that such polarising information can originate from a group of cells outside the tectum, namely, those which are subsequently to form the diencephalon. They also suggest that tectal cells do not acquire permanent positional labels before optic innervation. Detailed reports, including autoradiographic studies of histogenesis and electrophysiological evidence for the presence of normal synapses in rotated tecta, will be presented elsewhere.

Embryonic operation

The presumptive midbrain regions of 83 embryonic *Xenopus* were cut out and replaced after rotation by 180°. Most of the operations were carried out at stages 21-24, or approximately 22-26 h after fertilisation, with some at stage 37, some 24 h before the optic nerve reaches the tectum^{10,11}. No differentiation is detectable among cells of the neural tube until midway between these two periods, but earlier work on another species¹² has shown that even well before stage 20 the neural rudiment is a mosaic of regions, each with a strong tendency to develop into a particular part of the brain. We confirmed that this situation obtains also in *Xenopus* by examining embryonic brains following rotation of small pieces in neural plate stages.

The dorsal diencephalon (thalamus) and adjoining optic tecta develop from a dorsal section, some 15 to 20 cells square as counted directly through the dissecting microscope, of the stage 22 neural tube (Fig. 1). By varying the anterior-posterior levels of the cuts, but attempting to rotate in each case a similar sized piece, we were able to produce brains with different spatial arrangements of these anatomical parts.

Owing to elastic forces resulting from morphogenetic movements and cellular differentiation, the operation on the brain rudiment of stage 37 larvae is difficult. Nevertheless, we have succeeded in mapping two animals after rotating tectal structure at this stage.

We used several criteria for assessing operations as successful. First, we checked visually whether the cut tissue remained in place 48 h after operation. Second, we observed gross derangements of brain anatomy in cases where the rotated tissue had healed properly, and confirmed this in serial histological sections. Finally, when we carried out control experiments in which the cut piece of neural tube was discarded, we observed two classes of results from those animals surviving until metamorphosis. In one class of animals, regeneration had given rise to brain structure and retino-tectal connections indistinguishable from those of unoperated animals, and the other showed atectal brains with forebrain grossly enlarged and the diencephalon joining the brainstem.

Normal visual map in spite of rotated tectal tissue

Following the operation, only 10 animals failed to establish good retino-tectal connections. From the remaining animals, 63 electrophysiological maps of the direct optic projection to the contralateral tecta were obtained during metamorphic climax, using procedures detailed elsewhere¹⁰. For clarity, only one or two of the several rows of electrode positions plotted are shown for each map presented here. The brains and their associated maps fell into three main categories, which we now discuss in turn.

A parasagittal section of the normal brain is shown in Fig. 2a. The diencephalon (D) is seen immediately in front of the midbrain, with its single tectum (T). Figure 2b shows a comparable section of a brain where we believe the anterior part of the tectal precursor had been rotated. Viewed from the dorsal surface, a pair of tecta were clearly identifiable on each side, separated by the indentation and blood vessels seen at the tecto-diencephalic junction in the normal animal. In brain sections of such animals, tectal histogenesis can often be seen to progress from this new junction, forwards in anterior tectum and backwards, as normally¹⁰, in the posterior. As the recording electrode was moved successively backward from the anterior pole of the anterior tectum to the posterior pole of the posterior tectum, positions in the visual field from which electrical responses could be evoked also shifted from the anterior pole to the posterior pole. Note that, throughout, we use the terms anterior and posterior in relation to the animal's present body axis, rather than any original polarity of tissue in the embryo. Figure 3a shows the visuo-tectal map

obtained from one such animal, a histological section of which is exhibited in Fig. 2*b*. Similar anatomy and map were observed from two animals whose tectal rudiment had been rotated at stage 37 (Fig. 3*b*). Thus the array of

retinal fibres ignores the anatomical consequences of operation to produce a normal, single map with respect to the visual field and animal's body axis, by distributing itself across the two tecta. We therefore conclude that tectal tissue itself, rather than having assumed a fixed axial polarity for selective nerve connections, remains subject to overriding influences from outside the tectum until at least some 13 h after the equivalent retinal polarity is irrevocably determined²⁻³.

In many animals of the present category, particularly those where the new junctional groove and blood vessels were conspicuous between the anterior and posterior tecta, the path of the optic nerve was altered. Instead of entering the tectum at its anterior border¹³, the optic nerve now invaded both tecta from the position of the new intervening groove. Evidence for this is threefold. First, massive responses with large receptive fields were obtained at the junction point, instead of at anterior poles of both tectum and visual field as in normal animals¹⁰. Second, we observed instances in which both the anterior pole of the anterior tectum and posterior pole of the posterior one were electrically silent. Finally, histological preparations of certain animals clearly show ascent of fibres between the two tecta. We therefore believe that factors normally guiding these fibres to the tectum are distinctive to the diencephalon-midbrain junction, which has been transferred posteriorly in these operations. Regardless of the trajectory of the optic nerve, incoming fibres treated the two tecta as one and distributed themselves with correct order and polarity, thereby showing that control of the direction of innervation and of progressive tectal differentiation are quite independent of the polarity of the visuo-tectal map.

Reversal of visual maps related to position of diencephalon

When the positions of cuts, at operation, were brought forward by only some 5-10 cells in the neural tube, the diencephalic structure (epithalamus, thalamus and hypothalamus) developed behind the tectum, rather than in front (Fig. 4*a* and *b*). Thus a small group of precursor cells, at stage 24, are determined as diencephalic; they form the diencephalon even when moved to a position adjacent to hind-brain precursor cells. The anterior pole of the tectum in such animals was joined directly to the telencephalon, whereas normally the diencephalon is interposed between these two structures (compare Fig. 4*a* and *b*). A typical visuo-tectal map obtained from one such animal is shown in Fig. 4*c*. In contrast to a normal map, the anterior visual field was now represented in the posterior pole of the tectum, and the posterior in the anterior pole; the visuo-tectal map was completely reversed.

The medio-lateral polarity, unlike the anterior-posterior one, was not reversed. As in normal animals, fibres stemming from the ventral part of the retina (namely, those subserving the superior visual field, as the lens inverts the image) positioned themselves more medially than those from the dorsal retina. Because the original operation was symmetrical about the animal's midline, we do not expect, and indeed have never found, any reversal in the medio-lateral axis of maps.

Following the version of the operation just discussed, 13 animals developed two separate tecta on each side, separated by the diencephalon (Fig. 5*a*). This presumably occurred because of inevitable variability in the operation, whereby in these cases some tectal precursor tissue had been left unrotated posteriorly.

A parasagittal section of such an animal shows clearly that the infundibular recess and hypothalamic nuclei lie directly below the level at which the two tecta join, and the cellular mass constituting the dorsal and ventral thalamic nuclei is interposed between the tecta (Fig. 5*a*). In trans-

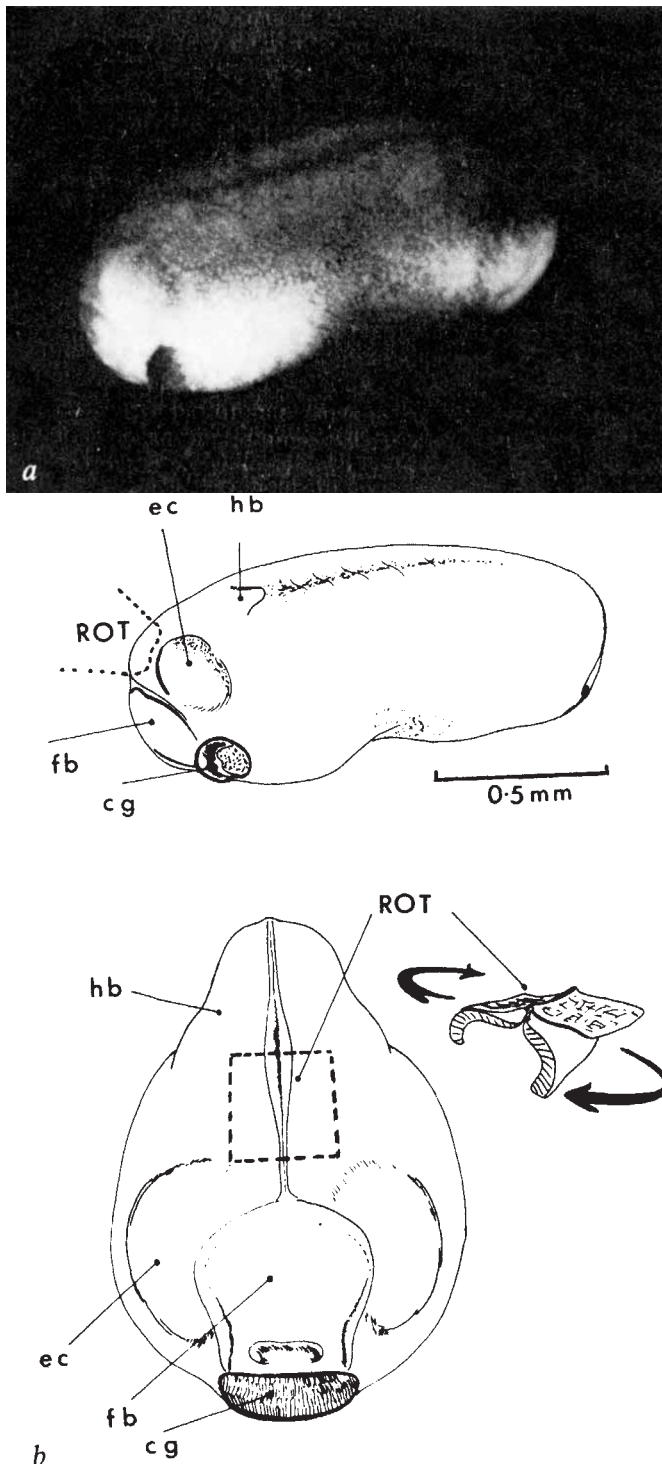


Fig. 1 Embryonic operation. *a*, Photomicrograph and camera lucida drawing of a stage 24 *Xenopus* embryo. Embryos were staged according to the tables of Nieuwkoop and Faber¹. Operations were performed with sharpened tungsten needles and hair loop, in two-thirds strength Niu Twitty solution brought to pH 7.1 with diluted HCl. On the drawings, the heavy dashed line marks the limits of tissue reversed in a typical operation. *b*, Drawing of the head from anterior-dorsal aspect at stage 24. ec, Eyecup; hb, hind-brain; cg, cement gland; fb, forebrain vesicle; ROT, rotated piece of neural tube and overlying epidermis.

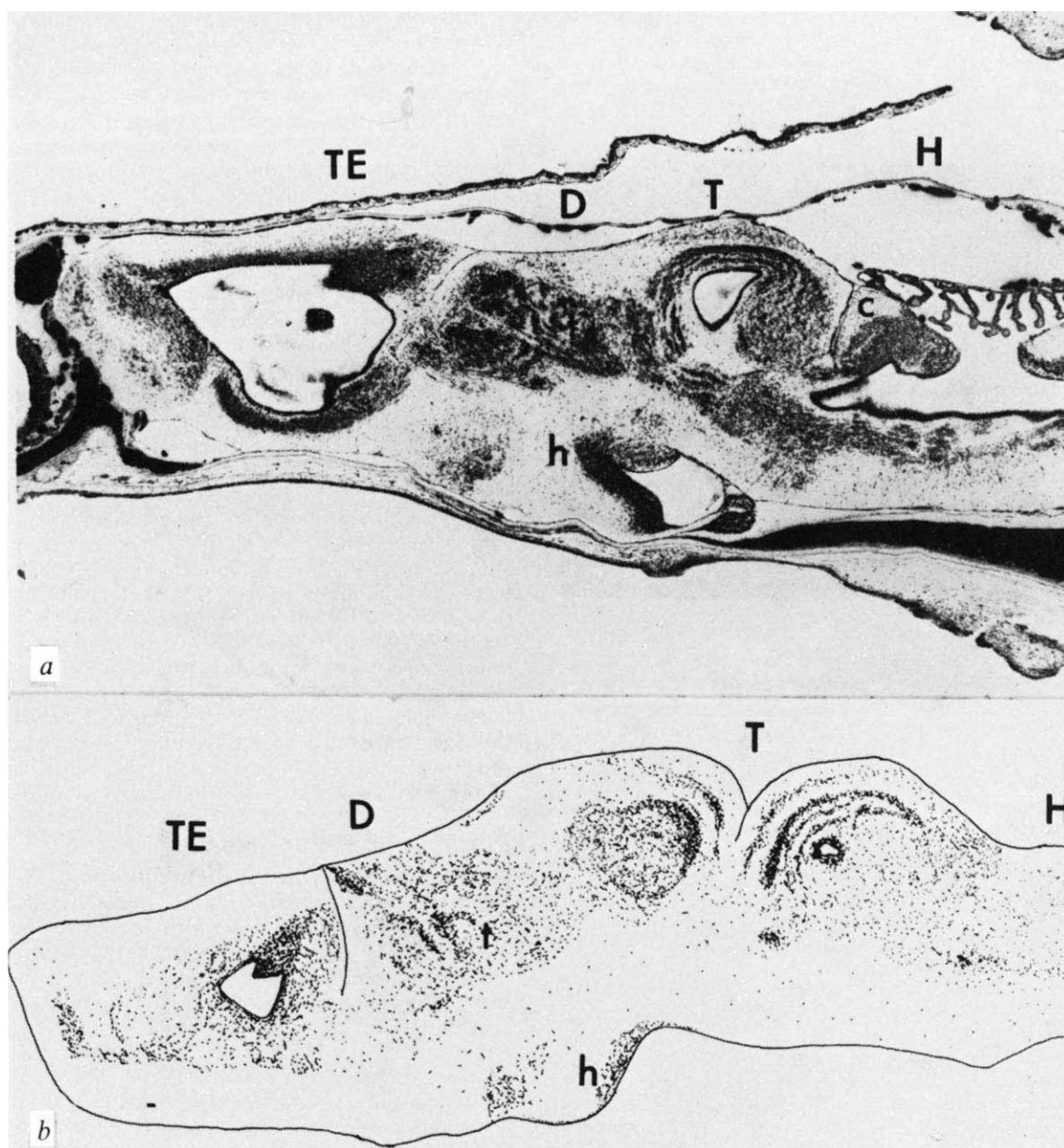


Fig. 2 Parasagittal sections of brains from (a) normal and (b) experimental tadpoles at stage 66. To make the rearranged structures more apparent, we have selected parasagittal sections throughout, although detailed anatomy is best followed in serial transverse sections. Because the experimental (b) animal was one also treated for ^3H -autoradiography, a *camera lucida* drawing is given since the visual appearance of the tectum is not comparable with the control (a) or with subsequent sections shown. TE, Telencephalon; D, diencephalon; T, tectum; H, hind-brain; h, hypothalamic nuclei; t, thalamus; c, cerebellum.

verse sections, not shown here, different diencephalic nuclei, including those of the epithalamus, can be identified more clearly. The pattern of visuo-tectal map in these animals showed that a complete visual field was represented in each tectum. The polarity of the map in the anterior tectum was reversed, whereas that of the posterior tectum was normal (Fig. 5b). Thus, the population of incoming fibres distributed themselves across the two tecta, those originating from the posterior part of the retina occupying, as in the normal brain, that tectal tissue adjacent to diencephalon.

In several animals, an extra diencephalon developed anterior to the tecta, in addition to the one between the two tecta. Embryologically, such an anomaly would be expected if some diencephalic cells were left *in situ* and some were translocated, and both groups then regulated to give complete structures. The anterior tectum, situated between the two diencephalons, showed two superimposed maps with opposing polarity, as illustrated in Fig. 6. When the recording electrode was at the most anterior pole of this

tectum, responses could be elicited both from the far anterior and far posterior parts of the visual field. Similar responses could be obtained from the posterior pole of this tectum. Two ordered series of symmetrical pairs of responses were then obtained as the electrode positions advanced along the intervening tectum. Such overlapping projections of opposing polarity indicate that each diencephalon can exert its own polarising influence on the intervening tectum, independently of the other.

Implications for selective nerve connections

Several inferences can be made from our study about the mechanisms governing formation of selective nerve connections. The most general conclusion we can draw from our findings, without recourse to any specific notions previously advanced, is that polarity for selective nerve connections within tissues remains labile long after that for the anatomical pattern of the brain as a whole is fixed. The possibility remains, however, that the same positional

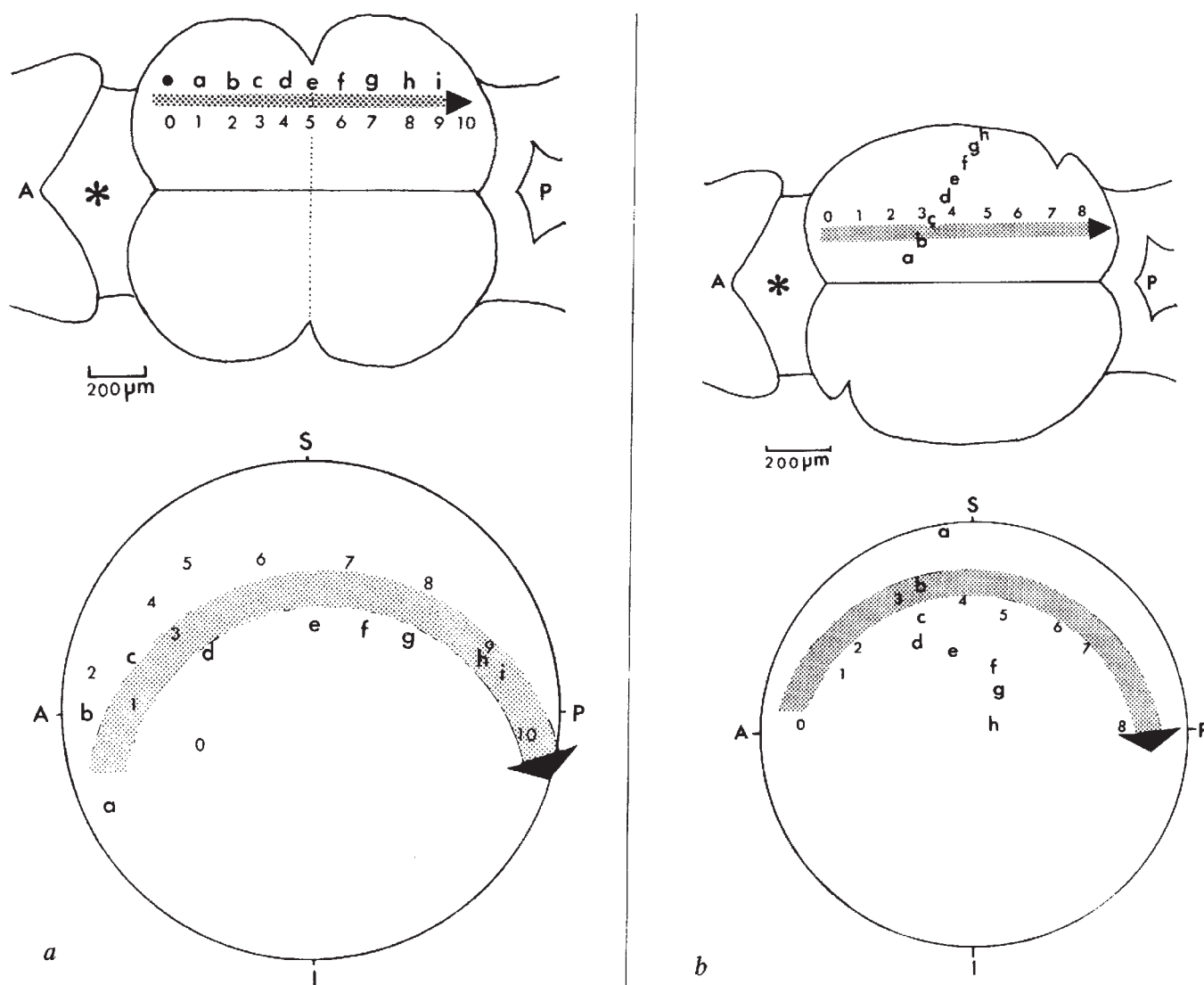


Fig. 3 Normal visual map after rotation of *a*, neural tube at stage 24 and *b*, tectal rudiment at stage 37. The map (*a*) was obtained from the animal whose histological section is displayed in Fig. 2*b*. All animals discussed in this paper were mapped between stages 62 and 66. Many rows of electrode positions were tested, but for the sake of simplicity, we show in this and following figures the results for only one or two rows. Positions in particular rows are either numbered or lettered consecutively, and positions in the visual field from which responses were obtained are then shown. Overall polarity of rows is shown for quick inspection by broad shaded arrows. Asterisks indicate the location of the diencephalic structure. A, Anterior; P, posterior; S, superior; I, inferior.

signal⁹ is involved first in morphological determination and subsequently in establishing axial reference for selective nerve connections. The nature of such signals and of the cellular responses to them remain unknown. Nevertheless, our findings provide a basis for experimental attack on these problems in developing nervous systems.

Our results also suggest that a group of cells other than the tectal precursors can organise axial information within the developing tectum for incoming optic nerve fibres. These cells appear to be some or all of those which are determined to form the diencephalic structure itself. This conclusion is strengthened by results of the first category of animals (Figs 2, 3 and 4). Polarity of visuo-tectal maps in these animals was normal, although tissue normally situated at the very junction of diencephalon and tectum had been transferred posteriorly. The diencephalon thus may act in a way quite analogous to an embryonic organiser, in that each of them controls an axial polarity in surrounding tissue. In the case of the diencephalon, the polarity is that for the retino-tectal connections. Classical organisers, on the other hand, control anatomical patterns of cellular differentiation in surrounding embryonic tissues^{9,14,15}. It is relevant to note

here that the organiser for the axial pattern of the whole body during amphibian development is a small group of cells at the dorsal lip of the blastopore, which later form the anterior mesoderm^{8,14,15}. This mesoderm then induces in overlying ectoderm the formation of the forebrain rudiment, of which the diencephalon is the central part. An obvious test for the hypothesis of diencephalic organiser is to transplant a presumptive diencephalon alone as a graft behind the presumptive tectum of the host. Our preliminary results from a series of such animals are conforming with our expectation.

Several models have been proposed for the formation of ordered retino-tectal connections during development^{8,10-18}. Before trying to evaluate these models, one has to ask a fundamental question: how much information is encoded in each tectal cell? Maximum information is assigned to each tectal cell if each possesses a uniquely labelled positional code⁶. On the other hand, the minimum possible information is assigned to the tectum if there is only one reference point within or outside it, to align properly the projection of incoming fibres, and no positional memory is encoded in individual tectal cells¹⁶.

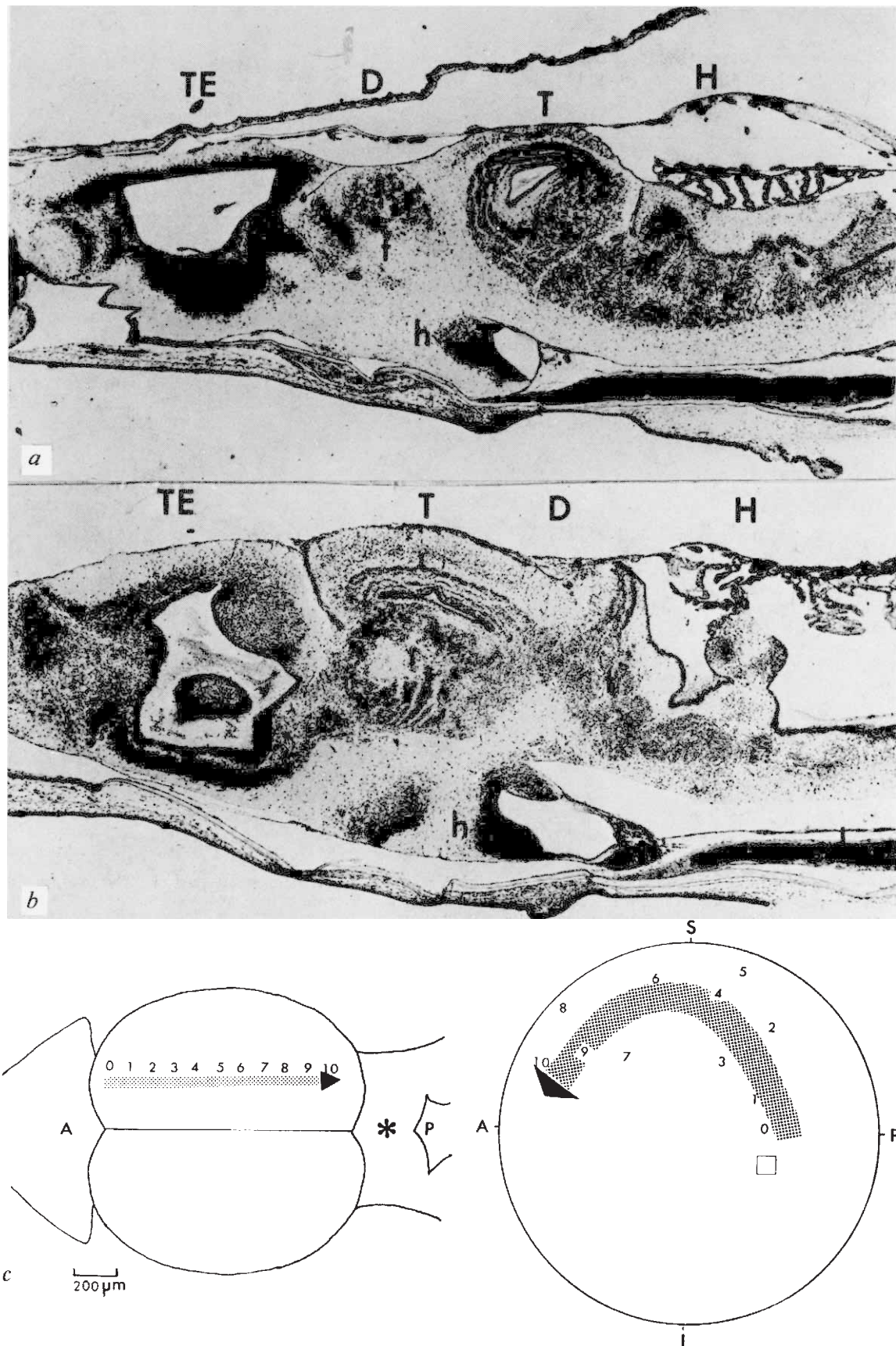


Fig. 4 Reversed visual map with diencephalon situated behind the tectum. Comparable parasagittal sections of *a*, normal and *b*, experimental animal (operated at stage 24). *c*, Reversed visuo-tectal map across the single tectum. The map and the histological section (*b*) were obtained from the same animal. From the electrode positions 2, 3, 4, 5, 6 and 8, responses could also be elicited by stimulating the visual field marked by the square. We can offer no plausible explanation for this anomaly. See Figs 2 and 3 for legend and abbreviations.

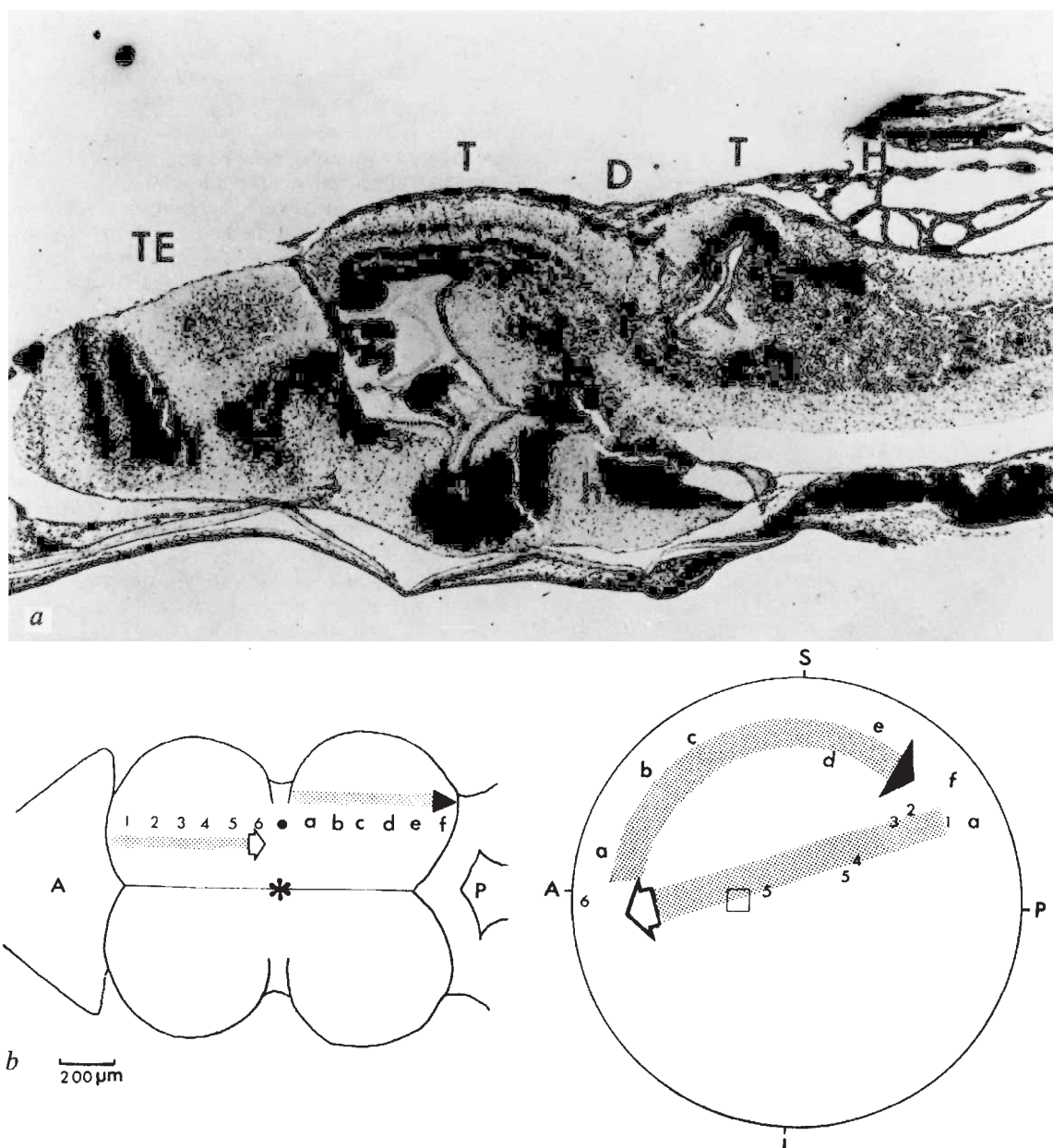


Fig. 5 Transposition of the diencephalon to lie between anterior and posterior tecta (operation at stage 24). *a*, Parasagittal section. *b*, Visuo-tectal map showing complete reversal for the anterior tectum and normal representation in the posterior tectum. The map and the histological section were obtained from the same animal. From the electrode positions 2, 4 and 6, responses could also be elicited by stimulating the visual field indicated by the square. See Figs 2 and 3 for legend and abbreviations.

Our present results show that positional labelling within tectal cells, if such ever occurs, can be overridden at least until stage 37 (Fig. 3*b*). Since functioning synapses are present by stage 45 in normal tadpoles¹⁹, any positional labels having significance in ordering connections must have been acquired within the intervening period. Because the tectal rudiment at such stages is fragile and sticky, we have not succeeded in eliminating this possibility. The observation of dual projection patterns with opposed polarity (Fig. 6), across the same tissue, nevertheless makes it difficult to suppose that tectal cells possess unique labels before optic innervation.

Our results are equally consistent with a theory which requires no positional memory in tectal cells. If we assume that, in addition to a reference point in the nervous system, there is a mechanism whereby optic nerve fibres can preserve, on the tectum, the spatial contiguity of their origin in the retina, a wide range of experimental data

including ours can be accounted for. The idea that mechanisms other than graded chemical labelling may be used by the nervous system in maintaining spatial contiguity, has been advanced previously. Lettvin's notion (cited in Chung¹⁶), for instance, is based on the fact that dendritic trees of retinal ganglion cells arborize extensively and interdigitate with neighbouring ones. Two fibres arising from adjoining retinal ganglion cells are, therefore, likely to exhibit a temporal contiguity in their discharge patterns, and such a coincidence in firing can, in principle at least, be used for maintaining spatial relations between the fibres. An alternative notion, advanced by Goodwin and Cohen²⁰, makes use of a signalling system arising from oscillatory metabolic processes in assigning positional codes to retinal and tectal elements, which then match up. In both of these models, each tectal element need not be uniquely labelled in order to establish ordered connections with retinal fibres. An underlying assumption in these

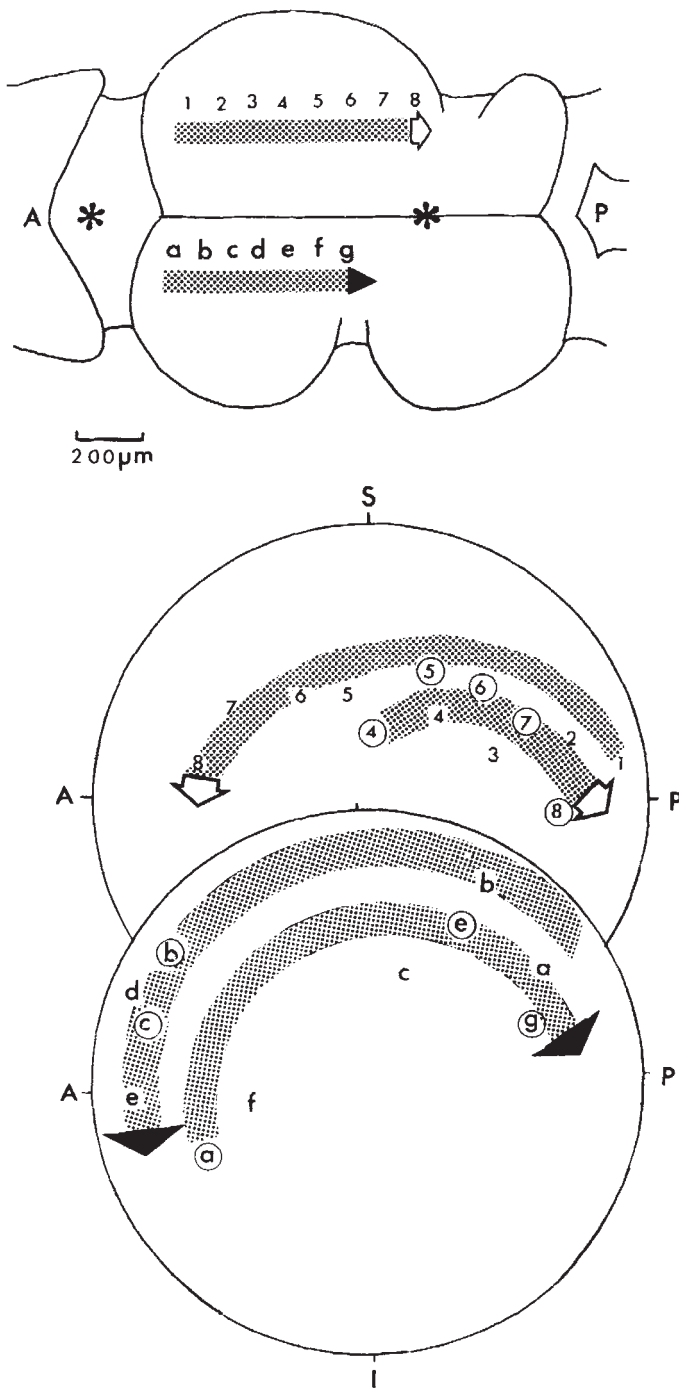


Fig. 6 Visuo-tectal maps obtained from tecta lying between 2 sets of diencephalic structure (operation at stage 24). Two superimposed ordered maps of the visual field with opposed polarity across the tectum, are seen on each side of the brain. Two circles represent the right and left visual fields. See text. For legend and abbreviations, see Fig. 3.

models is that fibres compete to achieve optimal matching with the available post-synaptic sites^{17,18}. Whether such models reflect physical reality remains to be investigated.

Experiments involving tectal rotation in adult *Xenopus*²¹ and goldfish^{22,23} as well as transplantation studies on the *Rana catesbiana* tectum^{24,25}, suggest that the situation in the adult brain may differ from that during development. For instance, when tissues from one part of the adult tectum were interchanged with those from another part, they appeared to retain memory of their previous position in 13 cases²³, whereas no such information was retained in 14 other cases²⁴. The conditions in which these two occur must first be ascertained.

A final evaluation of the two classes of theory we have outlined must, however, await further investigations. We propose that before tectal cells are innervated by optic nerve fibres, they possess no positional memory, and axial information for incoming optic fibres originates from a polarising structure located in the diencephalon. It can be shown either that critical events occurring between stages 37 and 45 irrevocably label tectal cells, or that a properly aligned map can be established in a developing tectum *in vitro* without diencephalic tissue, then our theory, in its simplest form, will have proved to be wrong.

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- 1 Nieuwkoop, P. D., and Faber, J., *Normal Tables of Xenopus laevis* (Daudin) (North-Holland, Amsterdam, 1956).
- 2 Stone, L. S., *Proc. Soc. exp. Biol. Med.*, **57**, 13-14 (1944).
- 3 Szekely, G., *Acta biol. Acad. Sci. Hung.*, **5**, 157-167 (1954).
- 4 Jacobson, M., *Dev. Biol.*, **17**, 202-218 (1968).
- 5 Hunt, R. K., and Jacobson, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 507-511 (1973).
- 6 Sperry, R. W., *J. comp. Neurol.*, **79**, 33-55 (1943); *J. Neurophysiol.*, **7**, 57-70 (1944).
- 7 Crelin, E. S., *J. exp. Zool.*, **120**, 547-577 (1952).
- 8 Spemann, H., *Embryonic Development and Induction* (Hafner, New York, 1967).
- 9 Wolpert, L., *J. theor. Biol.*, **25**, 1-48 (1969).
- 10 Gaze, R. M., Keating, M. J., and Chung, S. H., *Proc. R. Soc.*, **B185**, 301-330 (1974).
- 11 Chung, S. H., Stirling, R. V., and Gaze, R. M., *J. Embryol. exp. Morph.*, **33**, 915-940 (1975).
- 12 Jacobson, C.-O., *J. Embryol. exp. Morph.*, **7**, 1-21 (1959).
- 13 Potter, H. D., *J. comp. Neurol.*, **144**, 269-284 (1972).
- 14 Cooke, J., *J. Embryol. exp. Morph.*, **28**, 27-46 (1972); *ibid.*, **30**, 283-300 (1972).
- 15 Cooke, J., *A. Rev. Biophys. Bioengng.*, **4**, 185-217 (1975).
- 16 Chung, S. H., *Cell*, **3**, 201-205 (1974).
- 17 Gaze, R. M., and Keating, M. J., *Nature*, **237**, 375-378 (1972).
- 18 Prestige, M. C., and Willshaw, D. J., *Proc. R. Soc.*, **B190**, 77-98 (1975).
- 19 Chung, S. H., Keating, M. J., and Bliss, T. V. P., *Proc. R. Soc.*, **B187**, 449-459 (1974).
- 20 Goodwin, B. C., and Cohen, M. H., *J. theor. Biol.*, **25**, 49-107 (1969).
- 21 Levine, R. L., and Jacobson, M., *Expl Neurol.*, **43**, 527-538 (1974).
- 22 Sharma, S. C., and Gaze, R. M., *Arch. Ital. Biol.*, **109**, 357-366 (1971).
- 23 Yoon, M. G., *J. Physiol., Lond.*, **233**, 573-588 (1973).
- 24 Jacobson, M., and Levine, R. L., *Brain Res.*, **88**, 339-345 (1975).
- 25 Jacobson, M., and Levine, R. L., *Brain Res.*, **92**, 468-471 (1975).

letters to nature

Radio galaxies and local quasars

A RECENT discovery regarding Centaurus A (NGC5128)¹ may have an important bearing on the very local quasar model (see for example, refs 2 to 5). These observers, using the new 4-m telescope at Cerro Tololo Observatory, have obtained remarkable photographs showing visible filaments extending away radially from the galactic centre and blue starlike images never before seen near the Galaxy.

These starlike objects might have been called quasi-stellar had this term not taken on additional connotations since 1963, and this name may indeed be the proper one.

Such blue stars, presumably ejected from the galactic centre, are just what the very local quasar model predicts. They have been found in the area of the north-east radio lobe of Cen A at a distance of perhaps 80,000 light yr from the centre of that galaxy. If they have brightness similar to Type O stars, roughly 10,000 times brighter than the

Sun, they would appear as twelfth-magnitude stars as observed from the centre of NGC5128. Thus, to hypothetical astronomers in that galaxy, they would appear a little brighter than the quasar 3C273 as seen from Earth. These starlike objects near NGC5128 would then be of magnitude 23 or 24 as observed here, near the limit of observation.

If these blue stars are radio sources, as seems quite possible because of their location in the radio lobe, they could be producing radio emission by the same mechanism postulated for very local quasars²⁻⁵, that is, by relativistic motion through the gas and magnetic fields near the galaxy which ejected them. This would produce synchrotron radio emission in great strength and over a long time, drawing its power from the kinetic energy of the relativistically-moving star. It will be of considerable interest for radio astronomers to determine whether these blue objects are indeed radio sources.

In any case, the radio emission is coming from their vicinity, and the radio output and blueness give them some of the properties expected of local quasars—local to the galaxy NGC5128. If these objects are moving with relativistic speeds their proper motions would be $\sim 0.01'' \text{ yr}^{-1}$, and might just be observable over a number of years. Spectroscopy to determine the presence of a redshift or a blueshift would be very difficult, if not impossible, at this magnitude—near the limit of observation. Fluctuations in the optical and radio brightness would, however, be expected if these objects are indeed quasars local to Cen A, and should be looked for.

The energy required by the powerful radio emission from galaxies such as Cyg A and Cen A, which have strong emission from regions hundreds of thousands of light-years from the galactic centre, has been as much of a problem to explain as the energy source of quasars if cosmologically distant. Thus it may well be necessary to postulate the existence of very local quasars, massive objects thrown out (whether before or after condensation) from a gravitational collapse at the centre of a galaxy, in order to account for the puzzling properties of radio galaxies²⁻⁵. To inhabitants of those galaxies they would look much the same as quasars look to us, so that the explanation may well be the same in both cases.

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¹ Blanco, V. M., Graham, J. A., Lasker, B. M., and Osmer, P. S., *Astrophys. J. Lett.*, **198**, L63–L64 (1975).

² Terrell, J., *Science*, **145**, 918–919 (1964).

³ Terrell, J., *Science*, **156**, 265 (1967).

⁴ Terrell, J., *Nature*, **236**, 166 (1972).

⁵ Terrell, J., *Theories and Experiments in High Energy Physics (Proc. Orbis Scientia II, Coral Gables, January 1975)*, (edit. by Kursunoglu, B., Perlmutter, A., and Widmayer, S. M.), 457–473 (Plenum, New York, in the press).

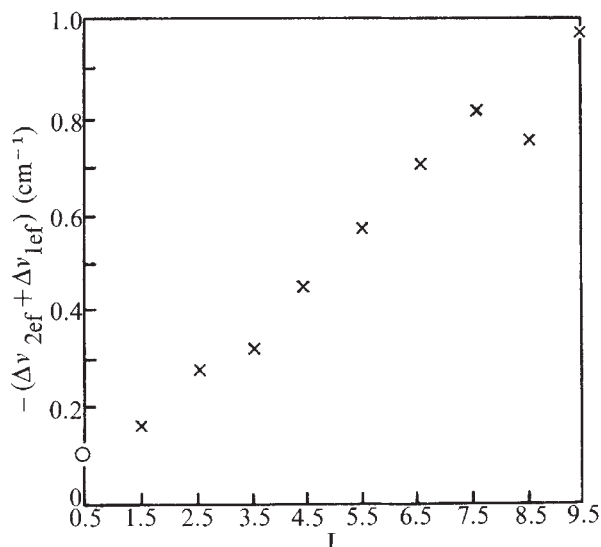


Fig. 1 Extrapolated experimental Λ -doublet splittings and the calculated value, experimental values of $-(\Delta v_{2ef} + \Delta v_{1ef})$ come from ref. 10, $\times$, experimental points; $\circ$, theoretical calculation for $J = \frac{1}{2}$.

Λ -doubling from high rotational levels populated in laboratory experiments to low rotational levels.⁴

Calculations are more difficult for SiH than for the similar CH species, since less is known about the perturbing excited states, and extra complications are introduced by the presence of a second row atom⁵. Nonetheless, predictions can be made if the approximations introduced by Van Vleck⁶ are used. To second order the splitting of energy levels of a $^2\Pi$ state may be expressed in terms of two parameters p and q , and the splitting in the lowest rotational level of a $^2\Pi_1$ state is given by $(\frac{1}{2}p + q)$, where:

$$p = 4 \sum \frac{\langle ^2\Pi | H_{so} | ^2\Sigma \rangle \langle ^2\Sigma | B(L^+ + L^-) | ^2\Pi \rangle}{E_{\Pi} - E_{\Sigma}}$$

$$q = 2 \sum \frac{|\langle ^2\Sigma | B(L^+ + L^-) | ^2\Pi \rangle|^2}{E_{\Pi} - E_{\Sigma}}$$

The summations extend over all vibrational levels of all $^2\Sigma$ states, with contributions from $^2\Sigma^-$ states being taken as negative. Matrix elements are taken over both electronic and vibrational wave functions; H_{so} is the spin-orbit coupling operator and B is the operator $\hbar/8\pi \mu r^2$.

Table 1 Λ -type doubling in the $X^2\Pi_1$ ($v = 0$, $J = \frac{1}{2}$) state of SiH

	$p(\text{cm}^{-1})$	$q(\text{cm}^{-1})$
Contribution from $B^2\Sigma^+$	0.02867	-0.00119
Contribution from $^2\Sigma^-$	0.05393	0.01273
Total contribution	0.08260	0.01154
Extrapolated experimental doubling	$2,940 \pm 300 \text{ MHz}$	
Calculated doubling	3,168 MHz	

Attention is restricted to the influence of the $B^2\Sigma^+$ state and an unobserved $^2\Sigma^-$ state. Electronic wave functions for the $X^2\Pi$ state and the $^2\Sigma$ states were obtained using the *ALCHEMY ab initio* wave function computer program⁷, including configuration interactions, using six configurations for the $X^2\Pi$ state, eight for the $B^2\Sigma^+$ state and five for the $^2\Sigma^-$ state.

Vibrational wave functions were obtained from the computed multiconfigurational potential curves for the $X^2\Pi$ and $B^2\Sigma^+$ states, but the $^2\Sigma^-$ state is found to be repulsive. For this latter state vibrational wave functions were obtained using the box normalisation technique of Gordan⁸ and Czarny *et al.*⁹ and the

Λ -type doubling in SiH molecule

IN recent years there has been much interest in the detection of the radio-frequency spectra of interstellar molecules. The observed spectra of the molecules OH and CH corresponded to transitions between the Λ -doubling components of the lower rotational levels. While experimental determinations proved very difficult, theoretical calculations on the Λ doubling in the lowest rotational level of CH have been notably successful^{1,2}. If the radioastronomical observation³ is corrected for the epicentre and the calculated value for centrifugal distortion, then the agreement of theory and experiment for the radioastronomical frequency is within 2 MHz in over 3,000 MHz.

SiH is similarly of interest as a probable interstellar species, but in this case the only available prediction for the radioastronomical frequency band must be made by extrapolating

contributions to p and q have to be integrated over the energy of the state.

The various contributions to p and q and the predicted doubling of the lowest level of $\text{SiH } ^2\Pi_{1/2}$ are given in Table 1. The figure shows the theoretical prediction compared with the experimental extrapolations. The agreement is close enough to provide some encouragement for a search for the molecule in interstellar space to be conducted, using available receivers.

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Received July 22; accepted September 17, 1975.

- ¹ Hammersley, R. E., and Richards, W. G., *Nature*, **251**, 597 (1974).
- ² Hammersley, R. E., and Richards, W. G., *Astrophys. J. Lett.*, **194**, L61 (1974).
- ³ Rydbeck, O. E. A., Eldér, I., and Irvine, W. M., *Nature*, **246**, 466 (1973).
- ⁴ Douglas, R. E., and Elliot, G. A., *Can. J. Phys.*, **43**, 496 (1965).
- ⁵ Walker, T. E. H., and Richards, W. G., *J. chem. Phys.*, **52**, 1311 (1970).
- ⁶ Van Vleck, J. H., *Phys. Rev.*, **33**, 467 (1929).
- ⁷ Bagus, P. E., in *Proc. Seminar Selected Top. Molec. Phys.* (IBM Germany, Ludwigburg, 1969).
- ⁸ Gordan, R. G., and Cashion, J. K., *J. chem. Phys.*, **44**, 1190 (1966).
- ⁹ Czarny, J., Felenbok, P., and Lefebvre-Brion, H., *J. Phys. B.*, **4**, 124 (1971).
- ¹⁰ Klynning, L., and Lindgren, B., *Ark. Fys.*, **33**, 73 (1966).

Haze in the stratosphere

ON September 1, 1975 I flew as a passenger on Concorde flight OF269 from Heathrow to Gander taking off shortly before 0730, arriving at Gander shortly before 1000 (London time). The return journey on flight OF277 started an hour later and arrived at Heathrow shortly before 1400. Over the Atlantic we climbed gradually from $\sim 50,000$ feet to $\sim 56,000$ feet.

I watched carefully for visual evidence of the tropopause throughout the flights. During the descent to Gander cirrus clouds extended up to 44,000 feet. There was occasionally slight clear air turbulence, sometimes regular enough to indicate a billow wavelength of ~ 120 m, at many different heights in the stratosphere, but most remarkable of all was the extent of haze layers.

The distant horizon was the cirrus cloud top which was variously at heights between 35,000 and 44,000 feet. Above that the sky had a whitish glow, and as we ascended, several discrete layers, in which the haze was denser, were revealed as we came to the top or bottom of the layer. When the sun was low, on the outward flight, they were brown coloured in the north-west; when the sun was high on the return flight at the eastern end, they glowed white in the south-east. I had a forward starboard window. The highest layers were still above the aircraft at 56,000 feet, and the sky glowed with a paler blue than on occasions when I have observed the colour of the stratosphere from jet aircraft, for example Comets, at $\sim 35,000$ feet. On this occasion the weather ship radiosondes indicated a very obvious tropopause at $\sim 39,000$ feet but it certainly did not stand out visually.

The haze extended all the way across the Atlantic, and its extent is very significant. We tend to think of haze as a cooling agent for the air, because that is what it is in normal low altitude pollution situations; but on this occasion it was a warming agent, being probably below the radiation equilibrium temperature between the Earth and outer space. Thus the presence of haze facilitates the ascent of air without adiabatic cooling in the stratosphere, and on this occasion could well have been associated with the exceptionally large descent taking place above hurricane Donna further south in the Atlantic.

The importance of this observation is that it makes nonsense of the assumption, implicit in almost all theories

concerning the consequences of putting pollution into the stratosphere, that practically everything that can, in the usual rainmaking processes, be rained out as air ascends into the stratosphere, actually is rained out and thus removed. This includes NO_x , sulphur compounds, such as SO_3 , and ammonium sulphate, and a large range of organic compounds of which the most important, in today's context of ozone depletion scares, is methyl chloride.

It is probably not necessary to look further than the normal natural interchange mechanisms across the tropopause for the greatest supply of all these substances to the stratosphere, and it is easy to stipulate that mother-of-pearl clouds are formed on layers of sulphate haze at heights around 80,000 feet. The implications of these observations make nonsense of the ozone depletion theories which depend absolutely on a degree of stratospheric purity that is quite unrealistic. Furthermore the ascent of such a vast extent of air on an occasion such as this makes nonsense of the supposition that the vertical transport mechanisms in the stratosphere can be correctly represented by diffusion mechanics in which all ascending chemical species mix and react chemically with all descending chemical species. Ozone depletion theories are based completely on diffusion models.

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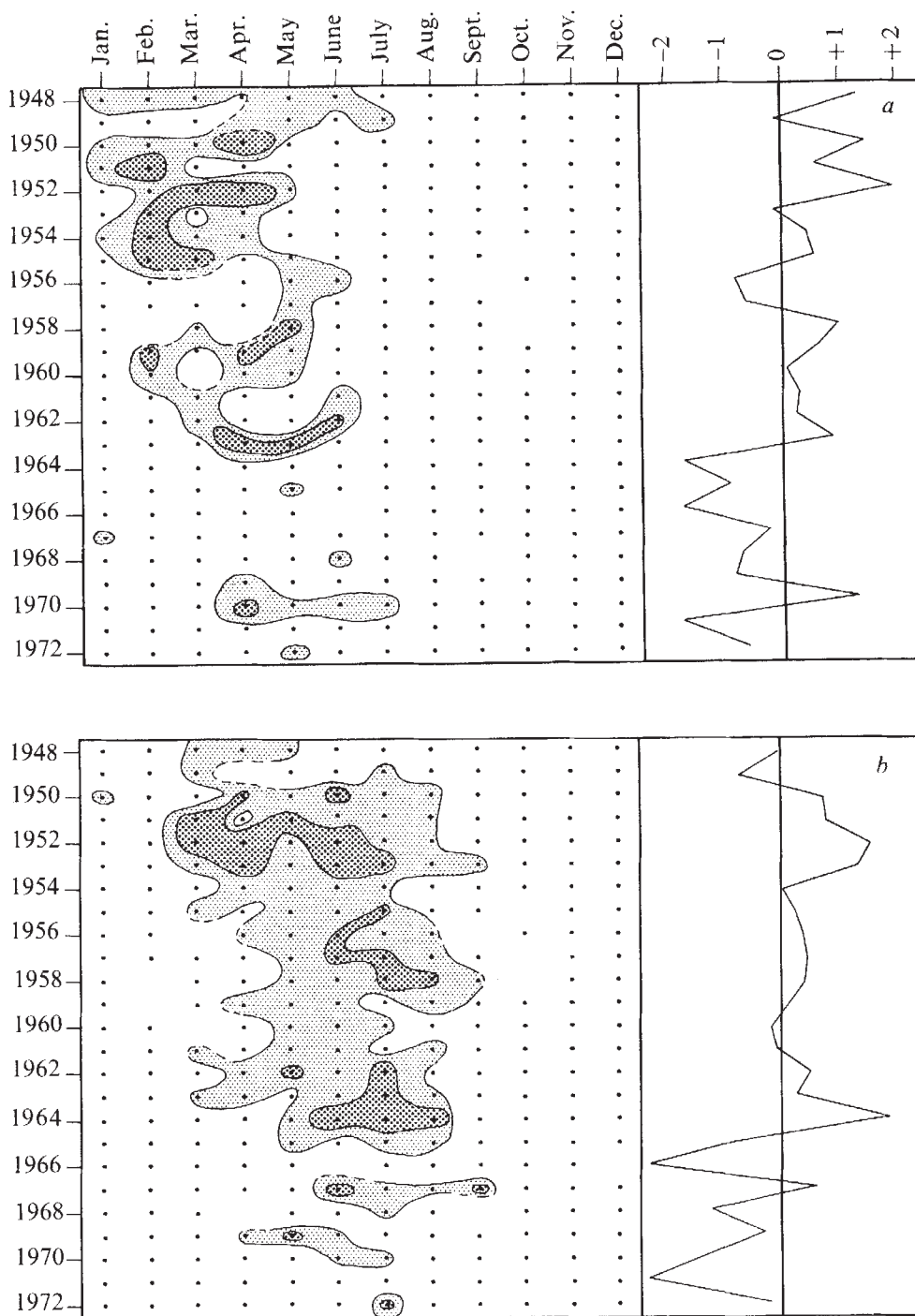
Continuous plankton records show fluctuations in larval fish abundance during 1948–72

THE continuous plankton recorder (CPR) survey¹ has sampled the plankton at a depth of 10 m around the British Isles consistently since 1948. Analysis of this material has revealed a number of trends^{2,3} in the distribution, abundance and timing of seasonal cycles of zooplankton and phytoplankton. This report discusses similar changes in the distribution and abundance of planktonic fish larvae.

Counts of fish larvae from selected recorder samples are allocated to statistical rectangles of 1° latitude by 2° longitude, and the mean number of fish larvae per recorder sample per month is calculated for each rectangle. These means are then used for the analysis of variability in distribution and abundance.

In the North Sea, at a depth of 10 m, there has been a decline in abundance of some larvae (for example *Odontogadus merlangus* (whiting), *Trisopterus esmarkii* (Norway pout), *Limanda limanda* (dab) and *Pleuronectes platessa* (plaice), and for some species (for example *O. merlangus*, *P. platessa* and *Ammodytes* spp. (sand eels)), a shortening of the season during which the larvae have been caught, and a delay in the time of the seasonal maximum abundance. Examples of these changes are given in Fig. 1, which shows monthly and annual fluctuations in the abundance of larvae of *O. merlangus* and *P. platessa* from recorder sampling in the North Sea. The larvae of both species declined in abundance over the period; the downward trend of *O. merlangus* started in 1964, whereas for *P. platessa* there has been a general trend over the whole 25-yr period. In both species the time when most larvae were sampled has shifted to later in the season and the season itself has shortened. These patterns bear a close resemblance to those found for the zooplankton and phytoplankton in the CPR survey; over the same period the total numbers of copepods and the zooplankton biomass in the North Sea have declined², the duration of the

Fig. 1 Monthly fluctuations in abundance of: *a*, *Pleuronectes platessa* and *b*, *Odontogadus merlangus* from 1948 to 1972, obtained from CPR sampling in the North Sea. Contour levels as mean numbers of fish per recorder sample are 0 and 0.05 for both diagrams; missing dots represent months with inadequate sampling. The right hand section of each diagram shows the fluctuations in annual abundance, standardised about a zero mean and plotted as standard deviation units. Annual abundance indices were obtained by summing monthly means.



zooplankton season has become progressively shorter², and the spring bloom of phytoplankton has become progressively later².

Similar changes have been recognised in CPR sampling of young fish in the English Channel and north-eastern Atlantic; charts showing the distribution of a common mid-water fish, *Stomias boa ferox* (dragonfish), for two periods are illustrated in Fig. 2. From 1948 to 1967 larvae were found only over deep water beyond the continental shelf, whereas from 1968 to 1972, the northern limit of distribution was much further south, and larvae were found over the shelf extending into the English Channel. These larvae had not hatched recently, so that their occurrence in the Channel was probably the result of drift from their spawning areas near the continental slope. Such an incursion of larvae into the Channel, observed since 1968, supports the hypothesis of an increased easterly flow of water through the Channel since about 1965, as proposed by Russell *et al.*⁴.

A southerly shift in the distribution of other fish larvae (like that of *Stomias* after 1967) is apparent from the CPR survey (for example *Melanogrammus aeglefinus* (haddock), *Micromesistius poutassou* (blue whiting)^{5,6}, *Scomber scombrus* (mackerel), and *Hippoglossoides platesssoides* (long rough dab)), and similar changes in the geographical distributions of fish^{4,7} and other marine animals^{4,8}, have been reported indicating an increased boreal influence from about 1965.

The seasonal period during which most of the *Stomias* larvae are found in the Western Approaches has shifted from March and April (between 1948 and 1961) to April, May and June (from 1968 to 1972). Since about 1965 there have been indications of a coincident shift in the main season of abundance of pilchard eggs in the Channel to later in the year⁴.

These changes in timing, abundance and distribution of fish larvae in the CPR survey add to the increasing evidence^{4,7,12} that many, perhaps most, pelagic organisms underwent a marked

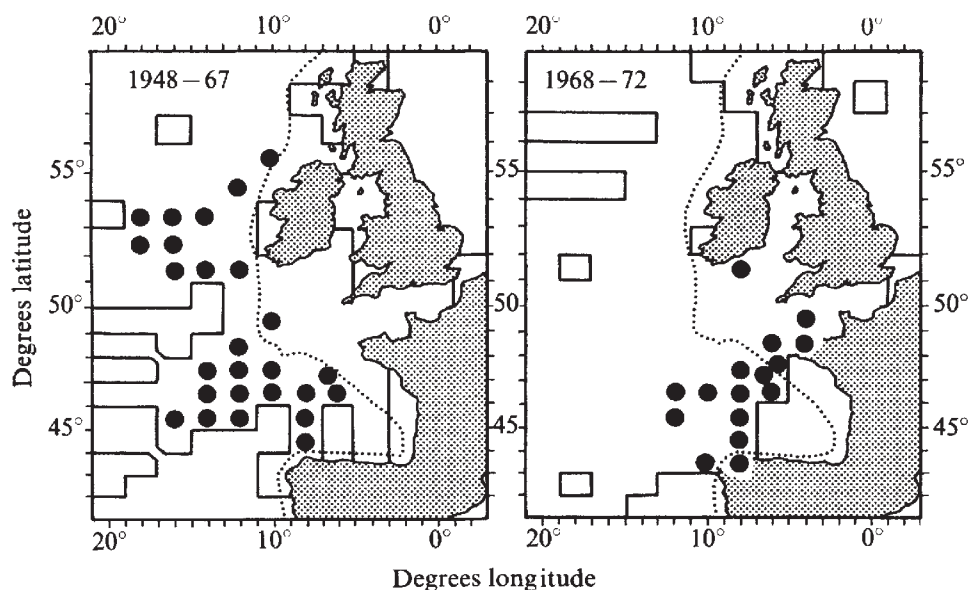


Fig. 2 The distribution of the larvae of *Stomias boa ferox*. Black dots indicate statistical rectangles in which larvae were found. The sampled area, from February to October, inclusive, is outlined, and the 200-m contour shown by a dotted line.

change in about 1965-67, in the waters of the north-western Atlantic and North Sea. Such a coherent and widespread pattern of change seems likely to have had its origin in climatic events^{9,10}. It is conceivable that climatic change may have affected the distribution of the adult fish, here reflected in the larval distribution, but it is also possible that the changes in the fish larvae have been caused by corresponding changes in the primary and secondary trophic levels, arising from the dependence of survival of young fish on the availability of prey organisms¹¹.

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- ¹ Glover, R. S., *Symp. zool. Soc. Lond.*, **19**, 189-210 (1967).
- ² Glover, R. S., Robinson, G. A., and Colebrook, J. M., in *Marine Pollution and Sea Life* (edit. by Ruivo, M.), 439-445 (Fishing News Books Ltd, London, 1972).
- ³ *Institute for Marine Environmental Research Report 1973-1974*, 49 (Edinburgh, 1974).
- ⁴ Russell, F. S., Southward, A. J., Boalch, G. T., and Butler, E. I., *Nature*, **234**, 468-470 (1971).
- ⁵ Bainbridge, V., and Cooper, G. A., *Bull. mar. Ecol.*, **8**, 99-114 (1973).
- ⁶ Coombs, S. H., *Cons. perm. Int. Explor. Mer.*, CM 1974/H: 35, 1-7 (mimeo).
- ⁷ Russell, F. S., *J. mar. biol. Ass. U.K.*, **53**, 347-355 (1973).
- ⁸ Southward, A. J., *J. mar. biol. Ass. U.K.*, **47**, 81-95 (1967).
- ⁹ Southward, A. J., Butler, E. I., and Pennycuik, L., *Nature*, **253**, 714-717 (1975).
- ¹⁰ Dickson, R., and Lee, A., *Fish. Indust. Rev.*, **2**, (2) 4-11 (1972).
- ¹¹ Cushing, D. H., in *Sea Fisheries Research* (edit. by Harden Jones, F. R.), 339-412 (Elek, London, 1974).
- ¹² Reid, P. C., *Nature*, **257**, 217-219 (1975).

Structure of graphite by neutron diffraction

THE structure of graphite¹ consists of layers of linked hexagons of carbon atoms. The layers are stacked in a ... ABAB ... sequence so that half the atoms in a layer are directly above and below carbon atoms in the adjoining layers, and half are directly above and below centres of the hexagons. The hexagonal unit cell contains atoms at the (0, 0, 1/4), (1/3, 2/3, 1/4), (0, 0, -1/4) and (2/3, 1/3, -1/4) positions. Modifications to this structure have been proposed by several workers. Lukesh² reported diffraction patterns indicating a lower symmetry, and Pauling³ has proposed an orthorhombic structure in

which the interconnected six-membered rings are distorted so that two-thirds of the bonds are longer. Pauling based his model on the high compressibility of the basal planes, and considerations of stacking bonds in the layered structure. Ergun⁴ suggested that data on pyrolytic graphite indicated an apparent quinoid structure in the graphite layers, but errors in his analysis have been reported⁵. X-ray and neutron diffraction studies of graphite were undertaken in our laboratory to study the details of the electron charge density and atomic motion in the graphite structure. The results of the neutron diffraction work show that all in plane carbon-carbon distances have the same value 1.422 ± 0.001 Å.

X-ray diffraction photographs of almost seventy small, natural single crystals of graphite indicated that two or three were of exceptional quality. The largest was a six-sided plate 0.2 mm thick, approximately 2 mm long and 1.4 mm across. This crystal was glued to a glass fibre, so that the axis of the fibre was parallel to the basal planes of the sample. Measurements were made on the full-circle automatic diffractometer at station H6S3 of the Brookhaven HFBR. A Ge (220) reflection was used to make the beam monochromatic, with wavelength of 1.02041 Å. Vertical in-pile soller slits and horizontal soller slits between the monochromator and sample were used. A $\frac{1}{2}'' \times \frac{1}{2}''$ receiving slit, 16'' from the sample and in front of the detector, was used. Twenty-seven reflections equally distributed over the diffracting sphere were centred to obtain accurate cell dimensions. A least-squares refinement of the angular positions of these reflections resulted in the unit cell dimensions and angles shown in Table 1. Assuming a triclinic cell, the *a* and *b* axes were equal to within σ (estimated standard deviation) and the angles between the unit cell vectors were 90°, 90°, and 120°, within 1-2 σ . Refinement assuming a hexagonal cell led to the lattice parameters also shown in Table 1.

Intensity data were obtained for 339 reflections, including all symmetry-related reflections within the 0.75-Å diffracting sphere. Step scans through each reflection were made in the

Table 1 Unit cell refinement

Triclinic cell	Hexagonal cell
$a = 2.464 \pm 0.002$ Å	$a = b = 2.464 \pm 0.002$ Å
$b = 2.466 \pm 0.002$ Å	$c = 6.711 \pm 0.004$ Å
$c = 6.711 \pm 0.004$ Å	
$\cos \alpha = 0.0003 \pm 0.0004$	
$\cos \beta = 0.0005 \pm 0.0003$	
$\cos \gamma = -0.5005 \pm 0.0003$	

Table 2 Graphite structure refinements

		P1 full sphere no restrictions	P $\bar{1}$ full sphere $z = 1/4$	P2 ₁ /m 1/4 sphere $z = 1/4, y = 1-x$	P6 ₃ /mmc 1/24 sphere (0, 0, 1/4), (1/3, 2/3, 1/4)
C ₁	<i>x</i>	0.0004 ± 0.0006	0	0	0
	<i>y</i>	0.0001 ± 0.0005	0	0	0
	<i>z</i>	0.2500 ± 0.0002	1/4	1/4	1/4
	<i>U</i> ₁₁	0.0031 Å ²	0.0031 Å ²	0.0031 Å ²	0.0031 Å ²
C ₂	<i>U</i> ₃₃	0.017 ± 0.001 Å ²	0.017 ± 0.001 Å ²	0.016 ± 0.001 Å ²	0.016 ± 0.002 Å ²
	<i>x</i>	0.3326 ± 0.0005	0.3325 ± 0.0004	0.3331 ± 0.0004	1/3
	<i>y</i>	0.6663 ± 0.0005	0.6664 ± 0.0004	0.6669	2/3
	<i>z</i>	0.2501 ± 0.0002	1/4	1/4	1/4
	<i>U</i> ₁₁	0.0031 Å ²	0.0031 Å ²	0.0031 Å ²	0.0031 Å ²
	<i>U</i> ₃₃	0.017 ± 0.001 Å ²	0.017 ± 0.001 Å ²	0.017 ± 0.001 Å ²	0.017 ± 0.002 Å ²
<i>R</i>		4.6%	4.6%	4.9%	4.2%

θ - 2θ mode with a scan width of 2θ of 4.5° - 6.0° and a step width of 0.06° . Of the 339 reflections measured, 52 would have zero intensity if the classical structure is correct. Of these 52 intensities, 38 were less than σ , 48 were less than 2σ and four could not be reduced because of uneven backgrounds or fluctuations. The standard deviations were based on counting statistics. No attempt was made to measure these reflections to a high degree of accuracy, and they were not used in the succeeding refinements. Examination of the intensity data indicated extinction effects were small and these corrections were neglected.

Thermal parameters of the form $\exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2hk\beta_{12} - 2hl\beta_{13} - 2kl\beta_{23})$ were used for C₁ and C₂. Initial refinements resulted in values of β_{11} , β_{22} , β_{12} , β_{13} and β_{23} that were smaller than estimated standard deviations for these parameters. Refinements setting these parameters equal to zero, and equal to values derived from other measurements of the mean square amplitude of vibration in the basal plane were carried out. No significant effects on the positional parameters were observed. The results of the refinements (Table 2) were obtained by assuming mean square amplitude of vibration of $0.0031 \text{ \AA}^2$ in the basal plane, based on neutron measurements by Ludsteck⁶.

Four full-matrix least-square refinements were done with varying restrictions on the structural parameters. In the first, a centre of symmetry was taken at the origin and no restriction was put on the positions of the two independent atoms in the unit cell, C₁ and C₂. The results of this refinement in space group P $\bar{1}$ indicated that the atoms in the basal planes are planar to within $\pm 0.001 \text{ \AA}$, and the classical structure of graphite is consistent to within 2σ . In the second refinement, it was assumed that the basal planes were truly planar, enabling one to fix the position of C₁ and refine only the *x*, *y* coordinates of C₂. The results of this refinement were not significantly different from the first. Ergun's proposed structure⁴ restricted the position of atom C₂ so that the *x*, *y* coordinates were related by $y = 1 - x$. The intensity data were averaged to a quarter sphere assuming

$$I(hkl) = I(\bar{h}\bar{k}\bar{l}) = I(hk\bar{l}) = I(\bar{h}k\bar{l}).$$

This refinement in space group P2₁/m provided further confirmation of the classical structure of graphite. If the Bernal structure¹ is assumed, only the thermal parameters are variables in the refinement. This refinement was done averaging the data to 1/24 of the sphere. Results of all four refinements are shown in Table 2.

The thermal parameters for C₁ and C₂ are not significantly different. The mean square amplitude of vibration perpendicular to the basal plane for C₂, which has voids above and below it, is nominally higher than that of C₁. These mean square amplitudes of vibration correspond to Debye temperatures of $490 \pm 40 \text{ K}$ and $470 \pm 40 \text{ K}$ for C₁ and C₂ respectively. These compare with Ludsteck's determination of the average

graphite Debye temperature for out-of-plane vibrations of $670^{+100}_{-70} \text{ K}$ (ref 6).

No indication of the proposed modifications of the graphite structure can be found in our data. Pauling predicted that two-thirds of the carbon-carbon bond distances may be $1.448 \text{ \AA}$ and one third $1.366 \text{ \AA}$. Comparable figures for the Ergun modification are $1.455 \text{ \AA}$ and $1.355 \text{ \AA}$. Our results show that all in-plane carbon-carbon bond distances are $1.422 \pm 0.001 \text{ \AA}$. It should be pointed out that this distance is averaged over time and over a large number of unit cells by the diffraction process.

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¹ Bernal, J. D., *Proc. R. Soc.*, **A106**, 749 (1924).

² Lukesh, J. S., *Phys. Rev.*, **80**, 226 (1950); **84**, 1068 (1951).

³ Pauling, L., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1646 (1966).

⁴ Ergun, S., *Nature phys. Sci.*, **241**, 65 (1973).

⁵ Donohue, J., *Nature*, **255**, 172 (1975).

⁶ Ludsteck, Von A., *Acta Crystallogr.*, **A28**, 59 (1972).

Photo-orientation of anisotropically absorbing molecules

THE action of plane-polarised light on anisotropically absorbing molecules produces an induced anisotropy in an ensemble of molecules, which can be detected by double refraction, dichroism and other optical phenomena. The phenomenon was first observed¹ on dye molecules in liquid², although its nature and mechanism by which it operates remain unexplained—neither can they be explained by the optical Kerr effect³.

Analysis of existing experimental material^{1,2} shows that there is a new kind of nonlinear optical phenomenon which takes place only upon the interaction of plane-polarised light with anisotropically absorbing molecules and which is resonant. Therefore, it can be observed at weak intensities using non-laser sources.

We suggest a classical model for an assembly of damped linear oscillators at resonance in a plane-polarised electric harmonic field. Each oscillator behaves according to the equation

$$m\ddot{x} + 2m\gamma\dot{x} + mf^2x = eE \cos\theta \cos\phi t \quad (1)$$

where m and e are the electron mass and charge, respectively, r is the damping coefficient, E is the amplitude of the electric field in an incident light beam, θ is the angle between the direction of the applied force and the axis of the oscillator, and f is the resonance frequency. The total energy of the oscillator is:

$$W(\theta) = (eE \cos \theta)^2 / 8mr^2 \quad (2)$$

The dependence on the angle θ leads to the appearance of a moment of force, $-(dW/d\theta)$, which tends to orient the oscillator perpendicularly to the direction of the electric field in a light beam. The anisotropy of the ensemble of mutually non-interacting oscillators at a given temperature, T , is subjected to the Boltzmann distribution

$$P(\theta) = C \exp(-W(\theta)/kT) = C \exp(-b \cos^2 \theta) \quad (3)$$

where $b = (eE)^2 / 8mr^2 kT$. Under plane-polarised resonance illumination the angular distribution of the oscillators becomes non-random. This photo-induced anisotropy causes several non-linear optical effects: deviation from Beer-Lambert law for plane-polarised light, photodichroism, and dependence on incident light intensity of the double refraction. Using the angular distribution function (3) these effects can be calculated with standard methods of statistical physics³.

The magnitude of the photo-orientation of anisotropically absorbing dye molecules can be evaluated at a weak field strength, 3 V cm^{-1} , which corresponds to the incident light intensities given in ref. 2. The damping coefficient, r , can be evaluated from the lifetime of the electronic excitation: $r = 1/\tau$. For dye molecules in a liquid, $\tau \sim 10^{-8} \div 10^{-9} \text{ s}$ (ref. 2). At room temperatures, when $\tau \sim 10^{-9} \text{ s}$ the nonlinearity coefficient b is $\sim 10^{-1}$. The corresponding value for the optical Kerr effect in such a weak field is $b_K = \alpha E^2 / 2kT \sim 10^{-13}$ (ref. 3). At resonance, the polarisability is $\alpha = e^2 / 4mfr$. Also, $b_K/b = r/f \sim 10^{-6}$. This explains the fact that photo-orientation induced by absorption was observed before the discovery of the laser and before the observation of the optical Kerr effect. The value of the nonlinearity coefficient being known, the photodichroism can be calculated using standard methods^{2,3}

$$d = (D_{\parallel} - D_{\perp}) / D = -0.5[\langle \cos^2 \theta \rangle - 0.5 \langle \sin^2 \theta \rangle] \approx b/15 \quad (4)$$

where D , $D_{\parallel}$, $D_{\perp}$ are, respectively, the optical densities of natural light, and light polarised parallel and perpendicular to the polarisation plane of the orienting light beam. If $b \sim 10^{-1}$, then $d \sim 10^{-2}$, which is of the same order as the experimental value of photodichroism observed by Neporent and Stolbova².

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¹ Teitel, A., *Naturwissenschaften*, **44**, 370 (1957).

² Neporent, B., and Stolbova, O., *Optika Spektrosk.*, **10**, 287 (1961); **14**, 624 (1963).

³ Schubert, M., and Wilhelm, B., *Einführung in die nichtlineare Optik* (Teubner Verlagsgesellschaft, Leipzig, 1971).

The electronic structure of VO^{2+} , CrO^{3+} , and MoO^{3+} complexes

THE electronic structures of the d^1 chromophores VO^{2+} , CrO^{3+} , and MoO^{3+} have been the subject of much debate¹⁻⁴, particularly after the early molecular orbital (m.o.) calculations of Gray *et al.*^{5,6}. An important feature of the electronic spectral assignments suggested^{5,6} was that the first two absorptions correspond to the d-d transitions $d_{xy} \rightarrow d_{xz, yz}$ and $d_{xy} \rightarrow d_{x^2-y^2}$, respectively, in the d-orbital sequence $d_{xy} (b_1) < d_{xz}, d_{yz}$

$(e) < d_{x^2-y^2} (b_1) < d_{z^2}(a_1)$. These assignments have found general acceptance and have formed the basis for the interpretation⁷ of the electron spin resonance (ESR) spectra and bonding parameters of these complexes. We report here experimental results contradicting the assignment of the second absorption.

We have obtained, for the first time, polarised single crystal electronic spectral data for CrO^{3+} and MoO^{3+} complexes of known structure, including $[(\text{C}_6\text{H}_5)_4\text{As}][\text{CrOCl}_4]$, $[\text{MoOCl}_3(\text{OP}(\text{N}(\text{CH}_3)_2)_3)_2]$, and $[(\text{C}_6\text{H}_5)_4\text{As}][\text{MoOCl}_4(\text{H}_2\text{O})]$, at room, liquid nitrogen and liquid helium temperatures. These data, together with certain features apparent^{3,4,8} for VO^{2+} complexes, show that several similarities exist between the electronic spectra of VO^{2+} , CrO^{3+} , and MoO^{3+} species (with the possible exception of fluoro-complexes).

(1) The energies of the first two absorptions are characteristic of the MO moiety and are essentially independent of the nature of the other ligands present. VO^{2+} complexes exhibit these absorptions at ~ 13 and 17 kK and MoO^{3+} complexes at ~ 13 and 22 kK . There are few spectral data available at present for CrO^{3+} complexes, but it is tentatively suggested that common absorptions occur at ~ 13 and 18 kK . These observations suggest that the second lowest energy absorption cannot be due to the $d_{xy} \rightarrow d_{x^2-y^2}$ transition as this should be very sensitive to the nature of the ligands *cis* to the M-O group.

(2) These two lowest energy absorptions are polarised. Both are allowed when the electric vector of the incident light is perpendicular to the M-O axis, and forbidden when it is parallel to this direction. Since C_{4v} symmetry is either exact or at least a close approximation for the actual structure of these chromophores, it follows that these two lowest energy electronic transitions must be $E \leftrightarrow A(B)$ in character. $d_{xy} \rightarrow d_{x^2-y^2}$ is a ${}^2B_2 \rightarrow {}^2B_1$ transition and is forbidden in both polarisations of C_{4v} symmetry.

(3) The sense of the polarisation and the absolute intensity of the first two absorptions remains essentially unchanged on cooling from room to liquid nitrogen and/or liquid helium temperatures. We therefore conclude that both transitions are electronically allowed. Although $d_{xy} \rightarrow d_{x^2-y^2}$ is symmetry forbidden, it could possibly give rise to an absorption of the observed polarisation by coupling with an E vibrational mode. If this were the case, however, a marked reduction in the intensity and the extent of the polarisation should be observed on cooling.

(4) Fine structure becomes apparent for these first two absorptions on cooling. In each case, the most common spacing of these details are $\sim 900 \text{ cm}^{-1}$ for the first absorption and $\sim 300 \text{ cm}^{-1}$ for the second.

To aid the interpretation of the electronic structure of these complexes we have performed *ab initio* m.o. calculations for the ground and excited states of certain CrO^{3+} and VO^{2+} complexes. These results indicate that the lowest energy absorption corresponds to the ${}^2B_2 \rightarrow {}^2E$ transition, $d_{xy} \rightarrow d_{xz, yz}$, or more correctly $M(d_{xy}) \rightarrow M-O(\pi^*)$. This interpretation is in agreement with the conclusions of Gray *et al.*^{5,6} and all the above experimental data. The fine structure associated with the transition is assigned to vibronic coupling with the M-O stretching mode of the first electronic excited state. In every instance the M-O stretching frequency seems to be less in the excited than in the ground state, as expected in view of the nature of the transition.

The experimental and theoretical evidence available at present for VO^{2+} , CrO^{3+} , and MoO^{3+} complexes gives definite guidance concerning the nature of the second lowest energy absorption, but does not allow for an unequivocal assignment. Self-consistent field calculations² for $[\text{CrOF}_5]^{2-}$ suggest that this absorption corresponds to a ${}^2B_2 \rightarrow {}^2E$ electronic rearrangement which is predominantly $M-O(\pi) \rightarrow M(d_{xy})$ in character, whereas configuration interaction calculations for $[\text{CrOCl}_4]^-$ suggest that the transition primarily involves the ${}^2B_2 \rightarrow {}^2E$ promotion, $M-O(\pi) \rightarrow M-O(\sigma^*)$, where the latter is the metal-ligand antibonding orbital involving d_{z^2} . Both of these assignments are consistent with points (1)-(4). The origin of the fine structure being attributed⁹ to vibronic coupling with the ML_{ctls}

B_1 stretching mode, this coupling being necessary⁹ to remove the excited state orbital degeneracy. Both suggested electronic transitions have excited state spatial degeneracy within orbitals localised essentially on the oxygen atom, where—in contrast to the first electronic transition where the excited state orbital degeneracy is concentrated on the metal centre—spin-orbit coupling effects are unlikely to remove adequately the spatial degeneracy. Since the calculated 2E excited state wave functions for the second lowest energy transition are an admixture of several electronic promotions, including the two cited above, it may not prove possible to give a simple description for the origin of this absorption.

The evidence we now have about the electronic structure of VO^{2+} , CrO^{3+} , and MoO^{3+} complexes clearly indicates a considerable similarity in their electronic structure. As generally assumed, the lowest energy absorption is essentially metal $d_{xy} \rightarrow d_{xz,yz}$ in character but the next absorption cannot be due to the $d_{xy} \rightarrow d_{x^2-y^2}$ transition to which it is usually attributed. This latter absorption seems to be due to an oxygen-metal charge-transfer rearrangement within the M-O group. This alternative interpretation destroys the basis used⁷ for the interpretation of the ESR spectra of these complexes and thus negates conclusions concerning the nature of bonding parameters so derived.

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¹ Ziebarth, O. V., and Selbin, J., *J. inorg. nucl. Chem.*, **32**, 849-865 (1970) and references therein.

² Garner, C. D., Hillier, I. H., Mabbs, F. E., and Guest, M. F., *Chem. Phys. Lett.*, **32**, 224-226 (1975) and references therein.

³ Selbin, J., *Chem. Rev.*, **65**, 153-175 (1965).

⁴ Robbins, D. J., Stillman, M. J., and Thompson, A. J., *J. Chem. Soc. Dalton Trans.*, 813-820 (1974) and references therein.

⁵ Ballhausen, C. J., and Gray, H. B., *Inorg. Chem.*, **1**, 111-122 (1962).

⁶ Hare, C. R., Bernal, I., and Gray, H. B., *Inorg. Chem.*, **1**, 831-835 (1962).

⁷ Manoharan, P. T., and Rogers, M. T., *J. chem. Phys.*, **49**, 5510-5518 (1968) and references therein.

⁸ Feltz, A., and Langbein, H., *J. inorg. nucl. Chem.*, **32**, 2951-2957 (1970) and references therein.

⁹ Ballhausen, C. J., *Introduction to Ligand Field Theory*, ch. 6 and 8 (McGraw-Hill, New York, 1962).

Singular behaviour of small crystallites near the melting point

DURING an extension of some recent *in situ* electron microscope investigations into the melting of small particles^{1,2}, some unexpected effects at temperatures near the melting point were observed for micron-sized crystallites of lead and bismuth. In particular, an unusually enhanced form of diffusion (termed 'snapping') occurred in which many crystallites changed their shapes abruptly into more compact solid forms. Experiments on somewhat smaller crystallites of tin have shown a similar behaviour. Changes of shape during the continuous deposition of thin films in an electron microscope have been reported by other workers^{3,4}; these, however, were of a more gradual character, and were consistent with normal diffusion processes.

A second phenomenon observed was the apparent bodily displacement (or 'crawling') of lead crystallites by distances comparable with their length. Small movements of the centre of mass of crystallites due to a change of shape have been noted by other workers⁵ and have also been observed in the present work, but these do not involve the

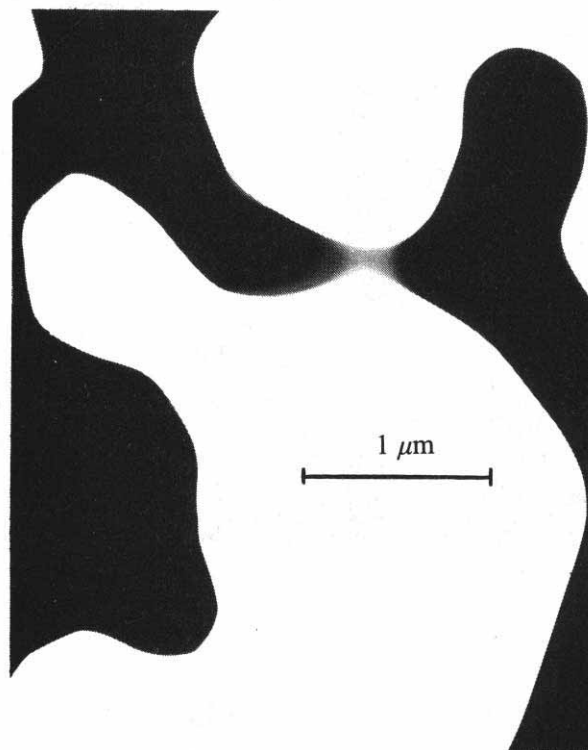


Fig. 1 The rupture, by atomic diffusion, of a neck formed in a thin film of lead (1-s exposure time). Although this diffusion is faster than usual, due to the high curvatures involved, the change is sufficiently slow to cause the edges to be blurred.

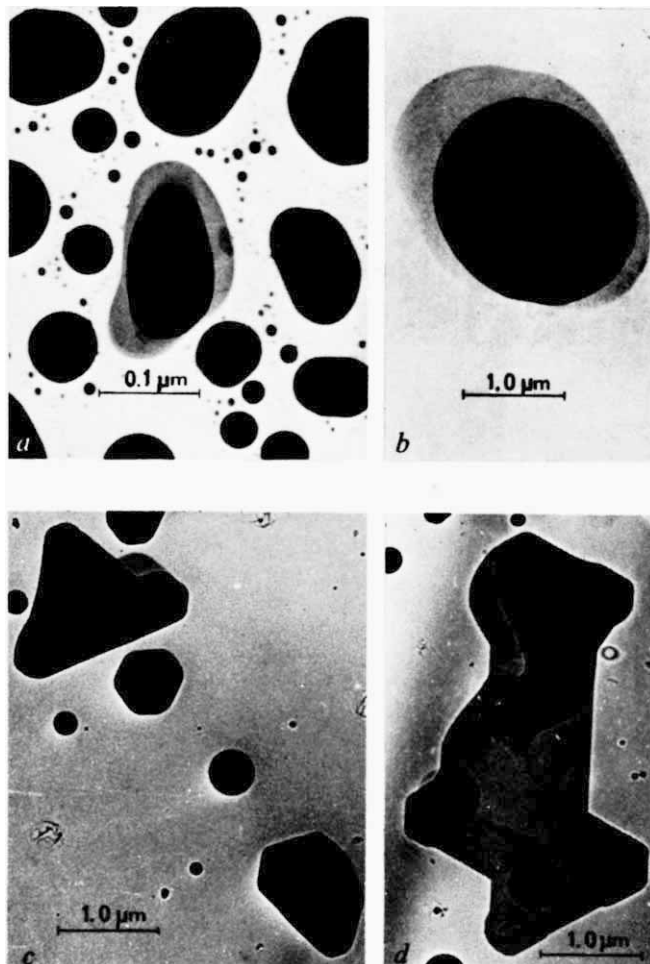


Fig. 2 Snapping in small crystallites (1-s exposure time). a, A tin crystallite; b, a lead crystallite, showing snapping and subsequent melting; c, bismuth particles; d, a bismuth crystallite snapping in to two solid and two liquid particles.

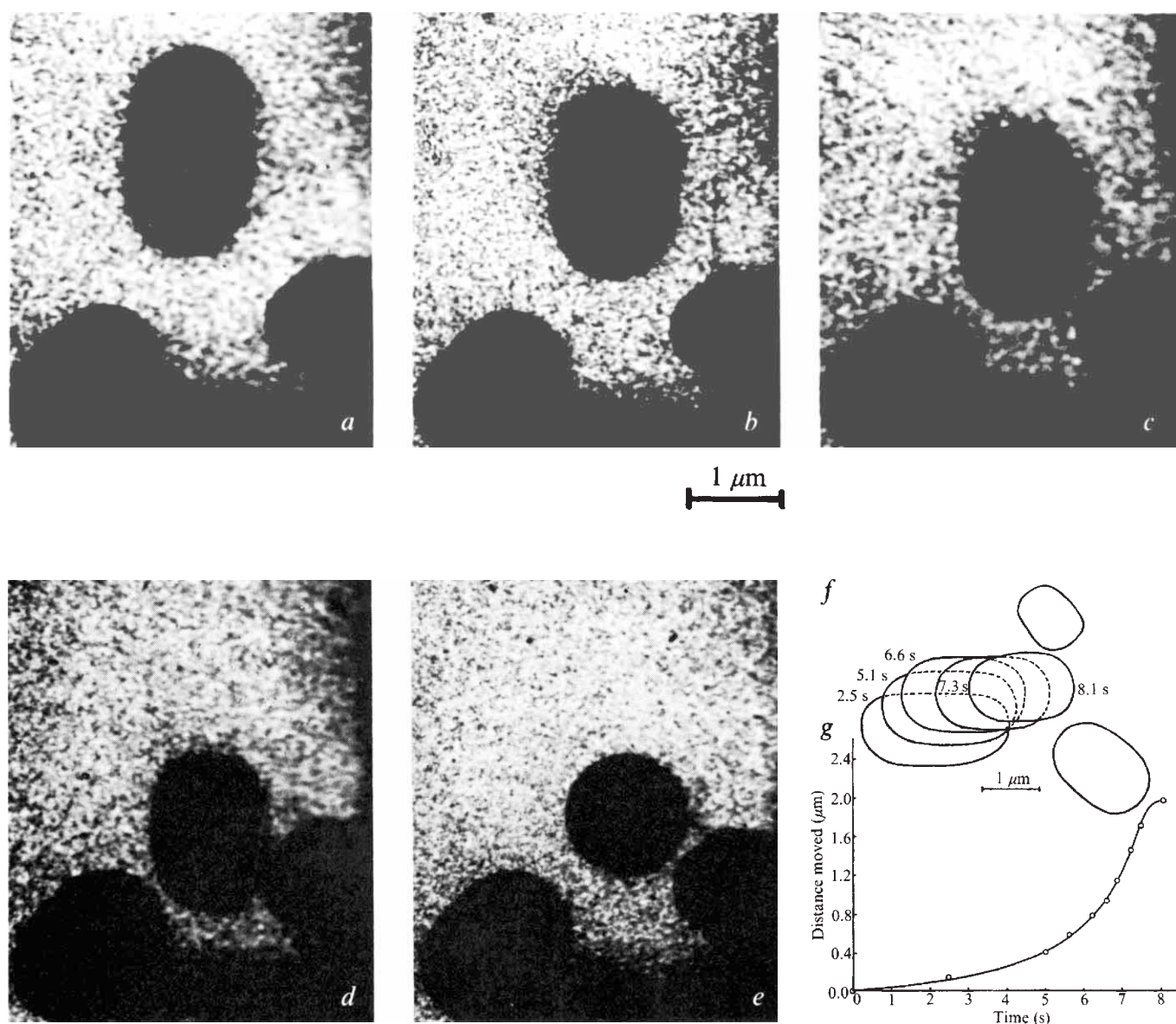


Fig. 3 *a–e*, Frames taken from a cine film showing the movement (and in the final frame the melting) of a lead crystallite relative to some stationary particles. The time with respect to the start of the moment in each case is: *a*, 5.1 s; *b*, 6.6 s; *c*, 7.3 s; *d*, 8.1 s; *e*, 15.6 s. *f*, Scale diagram showing the movement of the crystallite in Fig. 3*a–e*. *g*, Distance of movement plotted against time for the crystallite in Fig. 3*a–e*.

simultaneous movement of all parts of the crystal in a given direction. It is this fact which defines 'crawling' in the present work.

The experiments on bismuth and tin were performed in a JEM 6A electron microscope at a residual gas pressure of about 7×10^{-5} Pa (5×10^{-7} torr), and those on lead in a JEM 100U electron microscope at a residual gas pressure of about 10^{-5} Pa. The specimen films were evaporated *in situ*, usually on to amorphous carbon substrates of graphite, silicon monoxide and partially oxidised aluminium films. After deposition, the specimens were heated at rates ranging from 1 K min^{-1} to 10 K min^{-1} . In general the phenomena described were more pronounced at the faster heating rates. Records were made using either the normal internal plate camera, or a cine camera focused on the screen from a position outside the microscope.

Initially, the lead and bismuth deposits consisted of almost continuous films, containing narrow channels, while the tin deposits comprised individual irregular platelets. On heating, the first noticeable effect was the movement

of grain boundaries, resulting in an increase in the size of the grains. In the case of tin, this caused many of the particles to become single crystals, although they retained their irregular shapes. For the bismuth and the lead films, the channels increased in width because of atomic diffusion until the films formed first a network and then (usually within 40 K of the bulk melting point) individual, irregular platelets. This diffusion was slow except in regions of high (convex) curvature, particularly at points where, by the thinning of the material connecting two large particles, 'necks' formed and subsequently ruptured (Fig. 1). The resultant 'needle points' rounded off very rapidly at first, and then more slowly as shapes of lower curvature were formed. As expected, the rate of diffusion was observed to increase with temperature. Little diffusion of this type occurred in the specimens of tin which were already in the form of individual crystallites.

When the films were heated to within a few degrees of the melting point, very abrupt changes of crystal shape were observed to occur for all three materials; this effect was

independent of the base used. This was first noticed as the appearance of double images on micrographs taken during the experiments (Fig. 2). During the period of the exposure of the photographic plates (~ 1 s), some crystallites suddenly snapped from a thin form (the larger outlines in Fig. 2) to a more compact form. In other experiments, cine camera records showed no intermediate stage in this process, thus demonstrating the very rapid nature of the change—for example, in the case of bismuth, the change was completed in less than 0.04 s. This behaviour was not necessarily associated with particle forms differing markedly from equilibrium (as, for example, in the necking shown in Fig. 1).

The effect differed slightly for each of the three materials used. For tin (Fig. 2a) the change of shape was often symmetric; material from all the side faces building on to the top (and perhaps the bottom) surface. Occasionally, tin particles exhibited a double snapping in which two such abrupt changes occurred one after another, separated in time by ~ 1 s. For lead, the movement was also generally symmetric, but was often followed within a few seconds by melting (Fig. 2b). For bismuth the change was, as a rule, asymmetric with the product located towards one side of the initial crystallite. Usually one or more of the original side faces were preserved (Fig. 2c) and frequently the snapping resulted in the formation of two or more separated parts, some of which were often liquid droplets (Fig. 2d).

When heated to a temperature close to the bulk melting point, several of the lead crystallites were observed to crawl steadily on amorphous carbon substrates; during these movements there were gradual reductions in the projected areas of the particles, which became more compact. Figure 3a–c shows a sequence of photographs illustrating the crawling process, and Fig. 3f is an outline diagram showing part of the movement, in detail, of the crystallite relative to the surrounding stationary particles. The distance travelled by the centre of gravity of the particle shown in Fig. 3a–e has been plotted as a function of time in Fig. 3g. The movement of each crystallite generally lasted several seconds, and resulted in a total displacement roughly equal to the length of the particle. The crawling was observed at both high and low electron beam intensities, but was not seen in experiments performed on substrates of silicon monoxide, graphite and partially oxidised aluminium. Crawling was also absent on amorphous carbon in preliminary experiments carried out in a vacuum of about 7×10^{-5} Pa. The liquid droplets obtained after heating to above the bulk melting point showed no sign of such movements.

These experiments are being continued and will be reported in detail in due course.

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¹ Peppiatt, S. J., and Sambles, J. R., *Proc. R. Soc., A* **345**, 387 (1975).

² Peppiatt, S. J., *Proc. R. Soc., A* **345**, 401 (1975).

³ Pashley, D. W., Stowell, M. J., Jacobs, M. H., and Law, T. J., *Phil. Mag.*, **10**, 127 (1964).

⁴ Pashley, D. W., and Stowell, M. J., *J. Vac. Sci. Technol.*, **3**, 156 (1966).

⁵ Zhadanov, G. S., and Vertsner, V. N., *Kristallografiya*, **12**, 949 (1967).

Aerodynamic properties of an insect wing section and a smooth aerofoil compared

IN this communication I report measurements of lift and drag forces acting on corrugated aerofoils. The chordal profile of the wings in many orders of insects is corrugated¹ and I have used scale models of a wing section of this type found in the hover fly *Syrphus balteatus* (Diptera, Syrphidae). The experimental profile is shown in Fig. 1d. The life-size chord length is 2.9 mm, and occurs 3.7 mm distal to the wing-root hinge, in a total unilateral wing length of 11.5 mm. (There is no special reason either for selecting this particular section or for choosing *S. balteatus*.) The model profiles were 22 times life size (chord length = 6.4 cm) and were made of stainless steel 100 μ m thick. They were built to uniform chordal profile, with spans of 7.17 cm or 3.39 cm, giving aspect ratios of 1.12 and 0.53, respectively—both very low. An attempt was made to model the thick leading edge vein of the real wing by constructing the model leading edge from a stainless steel rod (diameter 1.7 mm).

Spanwise corrugation confers a large increase in spanwise stiffness (and allows a decrease in maximum membrane stress when the wing is subject to bending) by comparison with a flat plate of equal plane dimensions and mass. Because of the possibility that corrugations may be seriously detrimental to the aerodynamic qualities of a wing, the aerodynamic properties of the corrugated model were compared with those of another model of the same aspect ratio, of which the chordwise profile was that formed by drawing a smoothed 'envelope' through the corner points of the corrugated section. This is also shown in Fig. 1d, and was made of Perspex by grinding and polishing from a larger block.

The lift and drag properties of these models were tested in a small closed-circuit fluid flow channel filled with 45% aqueous

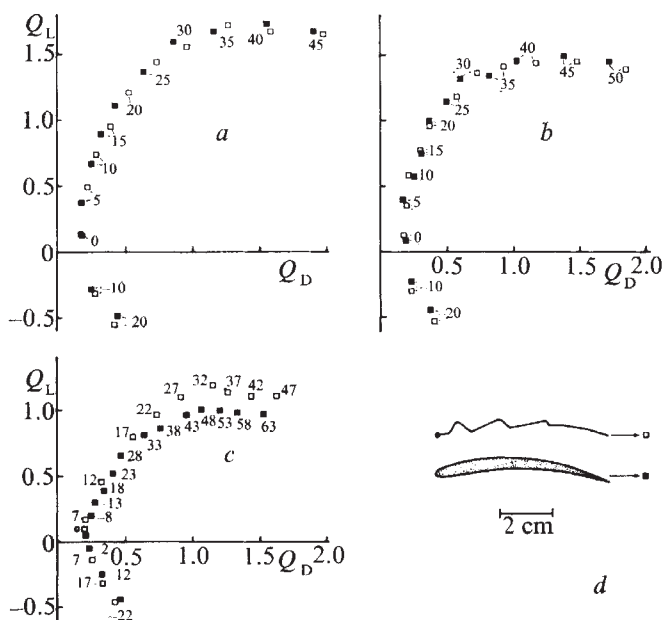


Fig. 1 a–c, Polar plots of quasi-lift coefficient Q_L against quasi-drag coefficient Q_D for the corrugated aerofoil ($\square$) and its envelope ($\blacksquare$). These profiles are shown in d. Small figures by each point refer to the angle of incidence at which that measurement was made. The use of the coefficients Q_L and Q_D and the experimental conditions in which they were measured are explained in the text. a, Reynolds number 900, aspect ratio 1.12; b, Reynolds number 450, aspect ratio 1.12; c, Reynolds number 800, aspect ratio 0.53; d, sections of corrugated aerofoil (stainless steel, 100 μ m thick, with 2-mm leading edge wire, $\square$) and its solid envelope (Perspex, $\blacksquare$). The corrugated chord section was modelled from a wing profile of the hoverfly *Syrphus balteatus* 3.7 mm distal to the wing root. (See ref. 1.)

sucrose solution. The external plan dimensions of the rectangular circuit were 120 cm × 70 cm, and the channel itself was 12 cm wide and was filled to a depth of 14 cm with the solution. The fluid was propelled round the channel by a 16-blade paddlewheel (diameter 60 cm) running in the long side of the channel opposite that containing the working section. This wheel was powered by a variable speed 500-W electric engine through a reduction gear and pulley system, so that free stream velocities in the range 4 to 25 cm s⁻¹ were available. Curved parallel plates were fitted in the channel at its circuit corners, and there were honeycombs just upstream of the working section, which was itself about 8 cm × 11 cm in size. Over this section, the worst photographically measured velocity variations (using very small bubble markers) were -0.4 and +0.2 cm s⁻¹ at a mean velocity of 8.4 cm s⁻¹, and -0.2 and +0.3 cm s⁻¹ at 19.8 cm s⁻¹. The temperature of the fluid was maintained at 20 °C, so that the kinematic viscosity of the fluid, ν , was 7.79×10^{-6} m² s⁻¹ (7.79 cStoke).

The wing sections were mounted on a force balance in which the transducers were two orthogonally bonded pairs of silicon strain gauges (Pye 2A3A120P). When one set of gauges was loaded in its direction of maximum deformation, the other pair recorded a small error signal. The extent of this non-independence was about +1.8% ('lift' as affecting 'drag' sensors) and +0.95% ('drag' as affecting 'lift' sensors), and the results were corrected for this. The wing models were immersed in the flowing fluid such that lift would act perpendicular to the stream in a direction parallel to the channel floor, and drag would act parallel with the stream and the channel floor.

Force measurements were carried out at Reynolds numbers of 900, 800 and 450. These were considered to be values approximately appropriate to the wing of this fly, estimated from relationships given by Weis Fogh², and the wing-beat frequency and stroke angle data of Sotavalta³. In free flow around an aerofoil, undisturbed by the presence of walls and fluid surfaces, the lift and drag coefficients C_L and C_D may be determined from the expressions:

$$C_L = 2L/\rho u^2 A$$

$$C_D = 2D/\rho u^2 A$$

where ρ is the fluid density, u is its free stream velocity, A is the projected surface area of one side of the aerofoil perpendicular to chord and span, and L and D are the forces of lift and drag acting on the wing. In as small a channel as that used here, it is inevitable that there will be interference from the walls and fluid surface, and so the calculated force coefficients should not be interpreted as absolute values. I shall therefore symbolise

these quasi-coefficients as Q_L and Q_D , and would stress that they apply to the flow system as described above, and are chiefly of interest from a comparative standpoint.

Conventional polar plots of Q_L against Q_D are shown in Fig. 1. The characteristics of corrugated and smooth envelope aerofoils are similar, especially in the comparisons with the models of higher aspect ratio. No abrupt stall is apparent from the plots at any of the Reynolds numbers, in fair agreement with Vogel's findings⁴ for *Drosophila* wings, although the values of Q_L are in general higher than his measurements of C_L . Breakaway was, however, observed at lower angles of attack in regions of the aerofoil away from the tips than near the tips themselves. Figure 2 shows fluid flow around the corrugated section, which has been made visible by means of hydrogen bubbles electrolytically released into the fluid, using a method similar to that of Schraub *et al.*⁵. When the flow near the tip was examined in this way, it appeared that high velocity fluid passing round the wing-tip vortex was preventing the stagnation of fluid in the boundary layer on the upper surface of the wing, and thus eliminating the tendency to stall, up to high (35°) angles of attack; this is seen in Fig. 2 for an angle of incidence of 32°. Breakaway was, however, simultaneously well established at lower angles of attack (about 25°) in regions near the centre of the span of the corrugated or envelope wing with the larger aspect ratio. Instability in flight due to stall will be felt most severely when it occurs at the wing tip. Perhaps the chance of a wing-tip stall is reduced by this form of injection of high velocity fluid, derived from the wing tip vortex. Flow over a corrugated wing seems to involve some fluid becoming trapped in the folds (B in Fig. 2), where it is either stagnant, or rotates slowly about an axis parallel to the span of the wing. The main flow seems to behave as if these folds were solidly infilled and as if the aerofoil were more like the 'envelope' profile.

It thus seems that this wing profile has, at Reynolds numbers of a few hundred, all the advantages of low mass, high stiffness and low membrane stresses in bending which are associated with corrugation, without any obvious aerodynamic shortcoming by comparison with the smooth profile. Perhaps this conclusion may apply also to other corrugated wings which work at Reynolds numbers sufficiently low for the corrugation to present no 'roughness' problem.

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¹ Rees, C. J. C., *Nature*, **256**, 200-203 (1975).

² Weis Fogh, T., *J. exp. Biol.*, **59**, 169-230 (1973).

³ Sotavalta, O., *Ann. zool. Soc. Fenn.*, **Vanamo**, **15**, 1-67 (1952).

⁴ Vogel, S., *J. exp. Biol.*, **46**, 431-443 (1967).

⁵ Schraub, F. A., Kline, S. J., Henry, J., Runstadler, P. W., Jr, and Littell, A., *J. bas. Engng*, **1965**, 429-444 (1965).

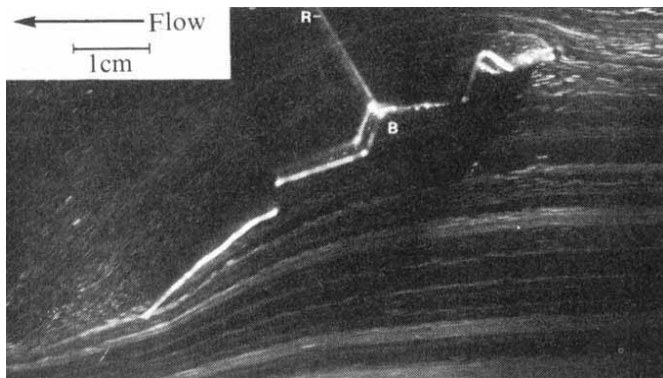
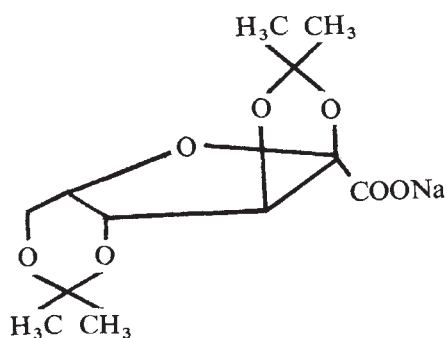


Fig. 2 Fluid flow around a corrugated aerofoil (same as used to obtain results in Fig. 1) made 'visible' by streaks, each representing the path of an illuminated hydrogen bubble during 1/30 s. Reynolds number 310. Bubbles were released electrolytically from wire upstream of the aerofoil. Fluid, sucrose 45% (w/v) + 0.04% NaCl; kinematic viscosity, 7.79×10^{-6} m² s⁻¹; aspect ratio, 0.53. R, Supporting wire (diameter 2 mm). B, Region where nearly stagnant fluid is trapped in fold. Incidence angle, 32°.

Group of new chemicals with plant growth regulatory activity

THE synthesis of L-ascorbic acid developed by Reichstein and Grüssner¹ is one of very few examples of large scale production of a biologically active monosaccharide. Although the importance of monosaccharides to plants has long been recognised, and several plant growth regulators have been discovered, few of these are carbohydrates and none is a monosaccharide. Active compounds which may be regarded as derivatives of monosaccharides, such as aminoglycosides², are chemically too complex to be synthesised on a large scale. We were therefore surprised to find that sodium 2,3:4,6-di-O-isopropylidene- α -L-xylo-2-hexulofuranosonate (I, accepted common name, dikegulac-sodium), an intermediate in the commercial synthesis of L-ascorbic acid, acts as a plant growth regulator.



(I)

To demonstrate the activity of this series of monosaccharide derivatives, Table 1 shows the growth-retarding activity of 11 compounds on *Poa pratensis* L. cv. Norma in the greenhouse. Water-soluble compounds were dissolved in water, and the others were formulated as wettable powders. A spray volume of 1,000 l ha⁻¹ and concentrations of 6,000 and 3,000 p.p.m. were used.

The salts were generally more active than the esters although the pentyl and methyl esters were also highly active. As the

Table 1 Growth retardation activity of dikegulac-sodium and analogues on Kentucky blue grass (*Poa pratensis* L.)

	Length 4 weeks after treatment (mm)	
	6,000 p.p.m.	3,000 p.p.m.
Untreated	197	40
-sodium salt	40	40
-ammonium salt	42	52
-calcium salt	48	53
-morpholinium salt	45	45
-methyl ester	48	52
4- <i>tert</i> -butylphenyl ester	98	155
-pentyl ester	48	48
-nonyl ester	53	53
2-propynyl ester	53	67
2-hydroxyethyl ester	43	58
4-cyanophenyl ester	65	123

$P = 0.05$; s.e. ± 7.0 .

sodium salt was the most active of the 11 compounds studied, its activity was tested in the greenhouse on a further 10 species (Table 2). Heights of *Lolium perenne* L. cv. Verna, *Digitaria sanguinalis* Scop., *Hordeum vulgare* L. cv. Union, *Malus sylvestris* Mill., *Vitis vinifera* L. Riesling. $\times$ Sylvaner and *Liqustrum vulgare* L. were recorded 4 weeks after treatment. The effect of dikegulac-sodium on petiole abscission was tested on *Phaseolus vulgaris* L. cv. Saxa. The plants were cut 1 cm from the main stem and lanolin paste containing the compound was applied to the cut ends. Abscised petioles were counted 6 d later. The effect on fruit ripening was tested on *Lycopersicum esculentum* Mill. cv. Rheinlandsruhm. The plants were sprayed when the first fruits turned red and ripe ones were counted 3 weeks later. Flowers on the first truss of *L. esculentum* Mill. cv. Tiny Tim plants were treated with the test solution using Luckwill's method³. Four weeks after treatment the fruits were cut and checked for seed production.

Table 2 shows the range of activity of dikegulac-sodium. Growth retardation was seen in a wide range of plants, for example, cereals, cultivated and weed grasses and woody plants. The retardation of *Digitaria sanguinalis* Scop. was sufficient for dikegulac-sodium to be considered herbicidal. The compound induced abscission and stimulated ripening, both of which have been reported to be associated with the plant hormone ethylene^{4,5}. The test compound also destroyed apical dominance in *Rhododendron simsii* Planch., inducing the production of axillary shoots and increasing the number of flowers on *Gerbera jamesonii* H. Bolus ex Hook. Dikegulac-sodium also induced parthenocarpic fruit set on *L. esculentum* Mill. cv. Tiny Tim.

Of all the monosaccharide derivatives tested so far in our laboratory, only the diketal analogues of 2-ketogluconic and 2-ketogulonic acids have shown interesting plant growth regulatory activity. Large scale tests have indicated that the most promising effects are the growth retardation and inhibition of apical dominance in woody plants, for example, *Liqustrum vulgare* L., *Thuja occidentalis* L. and *Rhododendron simsii* Planch. It is as yet too early to speculate on the impact which this group of compounds will have on plant production.

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Table 2 Growth regulatory activity of dikegulac-sodium on ten species

Test plants		Length (cm)	No. per plant	s.e.
<i>Hordeum vulgare</i> L.	Untreated	46.1		1.55
	6,000 p.p.m.	37.0		
<i>Lolium perenne</i> L.	Untreated	32.7		1.10
	6,000 p.p.m.	7.0		
<i>Digitaria sanguinalis</i> Scop.	Untreated	24.8		0.98
	6,000 p.p.m.	2.7		
<i>Malus sylvestris</i> Mill.	Untreated	25.2		1.02
	6,000 p.p.m.	15.3		
<i>Vitis vinifera</i> L.	Untreated	72.7		2.46
	1,000 p.p.m.	30.8		
<i>Liqustrum vulgare</i> L.	Untreated	27.3		0.29
	4,000 p.p.m.	12.5		
<i>Phaseolus vulgaris</i> L.	Untreated		0.5*	
	5,000 p.p.m.		1.2*	
<i>Lycopersicum esculentum</i> Mill.	Untreated		9.7†	2.17
	2,000 p.p.m.		16.3†	
<i>Gerbera jamesonii</i> H. Bolus ex Hook.	Untreated		4.6‡	0.37
	200 p.p.m.		6.6‡	
<i>Rhododendron simsii</i> Planch	Untreated		8.5§	0.56
	5,000 p.p.m.		13.2§	

* Abscised petioles.

† Ripe fruits.

‡ Flowers.

§ Side shoots.

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¹ Reichstein, T., and Grüssner, A., *Helv. Chim. Acta*, 17, 311 (1934).

² Umezawa, S., *Adv. Carb. Chem.*, 30, 111 (1974).

³ Luckwill, L. C., *J. hort. Sci.*, 24, 19-31 (1948).

⁴ Jackson, M. B., and Osborne, D. J., *Nature*, 225, 1019 (1970).

⁵ Burg, S. P., and Burg, E. A., *Bot. Gaz. (Chicago)*, 126, 200 (1965).

Induction of hormone sensitivity by dehydration is one positive role for drying in cereal seed

THE hydrolysis of food reserves in seeds (usually fats, starch and protein) is closely linked to embryo growth since it does not begin until germination has started. The control of food mobilisation by the embryo is best understood in cereals such as barley and wheat. In these cases the hormone gibberellin, moving from the embryo, activates the aleurone layer to produce an array of hydrolytic enzymes, including α -amylase, protease and β -1,3-glucanase and to secrete them into the endosperm where stored materials are located¹. The stored food is of course laid down in the grain during its development on the mother plant. But at the time when this accumulation is taking place the grains contain relatively high levels of gibberellin²; nevertheless, the food reserves seem free from hydrolytic attack. One possible reason for this is that in undried grains on the mother plant the hormonal control system for enzyme synthesis and secretion does not function. No research has been done directly on this problem; what relevant research there is shows some discrepancies^{3,4}. Our results, described here, demonstrate that the hormonal control system operates fully only after the mature grains have been dried. Dehydration is necessary for the development of complete sensitivity to gibberellin.

Barley (*Hordeum distichum* cv. Julia) was grown in the greenhouse from December 1974 to March 1975 with supplementary lighting to give a 16-h daily light period, or in the field at the Lord Rank Research Centre, High Wycombe. Grains were collected approximately 30 d after anthesis. The grains were dehulled (fresh weight 60 ± 3 mg per seed) and half the sample was left to dry at room temperature for 2 d when a weight of approximately 31 mg per seed was reached (a water content of 9.6%). Embryos were removed from the dried and undried samples and the embryoless grains were sterilised in sodium hypochlorite (1.4% available chlorine) for 20 min. They were then rinsed several times and incubated according to the method of Jones and Varner⁵ but with the medium containing chloramphenicol ($10 \mu\text{g ml}^{-1}$) and nystatin (100 U ml^{-1}). Gibberellic acid ($10^{-6} \text{ mol l}^{-1}$) was included in the flasks. Incubation was terminated at different times and the medium was assayed for α -amylase⁵ and β -1,3-glucanase⁶. These enzymes were chosen because α -amylase is both synthesised and secreted in response to gibberellic acid, whereas the β -1,3-glucanase requires the hormone only for its secretion. Thus, the two hormone-controlled processes, enzyme synthesis and enzyme secretion, could be studied separately. The aleurone layers were removed from the grains without embryos for extraction of any non-secreted enzymes remaining in the cells.

The results for α -amylase (Fig. 1) show the characteristic hormone-induced enzymatic response in the case of the previously dried grain, but the undried grains fail to respond until after extremely long exposure to gibberellic acid and then to only a small degree. Increased response by undried grains

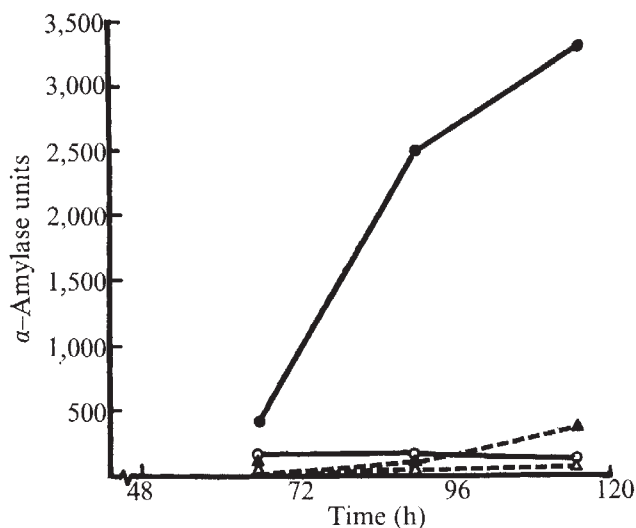


Fig. 1 α -Amylase production by dried and undried half grains. Ten half grains were incubated for appropriate lengths of time in 2 ml of medium. After incubation, medium was decanted, half grains were washed with 2 ml of buffer and washings were added to medium. After centrifugation supernatant was assayed for secreted enzyme. Aleurones were removed from washed half grains and homogenised in 4 ml of buffer. After centrifugation supernatant was assayed for non-secreted enzyme. —, Dried grains; ---, undried grains; ● and ▲, secreted enzyme; ○ and △, non-secreted enzyme.

can, however, be provoked by very high gibberellin concentrations but even so, enzyme synthesis and secretion at the level exhibited by dried seeds is never attained (Table 1). The degree of sensitivity of barley aleurone cells to gibberellin therefore clearly depends on previous dehydration of the grain. The findings suggest that there would be no appreciable breakdown of starch during its accumulation in the developing grain as the hormonal control system for enzyme production would be inoperative. The biological importance of this is clear. The practical implications are connected with the spoilage of dried cereal grain when this is rewetted before harvesting; here, hormone-controlled enzyme production can occur, leading to starch hydrolysis.

The pattern for β -1,3-glucanase is different, however, since dried and undried grain show only slight differences in total enzyme (Fig. 2) but drying does have some relatively small effect on the gibberellin-sensitive secretory mechanism. Thus the indication is that secretion can function almost fully even in seeds which have not been dehydrated. The results for

Fig. 2 β -1,3-Glucanase production by dried and undried half grains. Half grains were incubated and enzymes extracted as described for Fig. 1. Symbols as in Fig. 1.

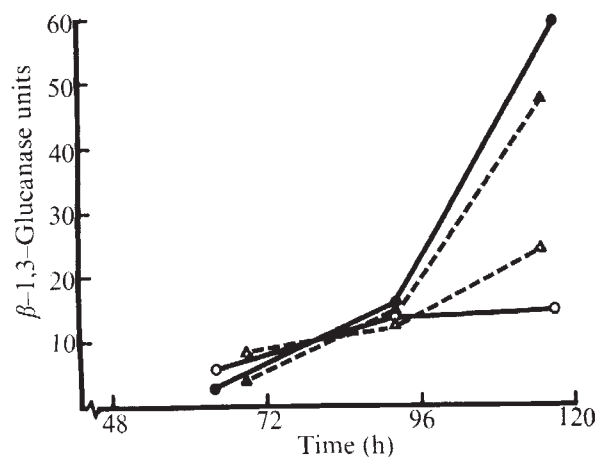


Table 1 Effect of gibberellic acid concentrations on α -amylase secretion in undried grains

Gibberellic acid concentration (mol l ⁻¹)	α -Amylase units (per 10 embryoless grains)
10 ⁻⁶	420
10 ⁻⁵	900
10 ⁻⁴	1,000

Ten half grains were incubated for 114 h in 2 ml of medium containing various concentrations of gibberellic acid. After incubation, medium was decanted, half grains were washed with 2 ml of buffer and washings were added to medium. After centrifugation, the supernatant was assayed for enzyme activity.

α -amylase and β -1,3-glucanase together indicate that previous drying is required probably only for hormone-induced synthesis (for example, α -amylase) and not for enzyme secretion.

The control of hormonal sensitivity by dehydration is matched by the effect of this treatment on germination itself⁷. In our experience undried, intact grains as collected for the above experiments do not germinate, but when dried for 2 d the rewetted seeds germinate within only 3 d at 20 °C.

In all, our results demonstrate the critical importance and positive role of dehydration in the physiology of a cereal seed. These findings demonstrate (apparently for the first time) how the full operation of a hormonal system in a plant tissue is instigated by previous drying. As far as the biology of seeds is concerned we should see this as a device for retaining the integrity of the food reserves until dispersal from the mother plant has been achieved. Together with this, the continued growth of the embryo (that is, germination) is restrained and can recommence only after drying has been experienced. The physiology of these two aspects of the controlling role of dehydration may be basically the same, connected with the regulation of the synthesis of enzymes, in one case for food reserve hydrolysis, in the other for germination itself. Experiments are in progress to investigate these phenomena further.

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- Briggs, D. E., in *Biosynthesis and its Control in Plants* (edit. by Millborrow, B. V.), 219–273 (Academic, London and New York, 1973).
- Rejowski, A., *Bull. Acad. pol. Sci. Cl. II Ser. Sci. biol.*, 17, 641–644 (1969).
- Duffus, C. M., *Phytochemistry*, 8, 1205–1209 (1969).
- Bilderback, D. E., *Pl. Physiol. Lancaster*, 48, 331–334 (1971).
- Jones, R. L., and Varner, J. E., *Planta*, 72, 155–161 (1967).
- Taiz, L., and Jones, R. L., *Planta*, 92, 73–84 (1970).
- Sprague, G. F., *J. Am. Soc. Agron.*, 28, 472–478 (1936).

Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone

THE maturation of spermatozoa during passage through the mammalian epididymis, involves changes in motility, metabolism, morphology, biochemical properties and the development of the ability to fertilise ova¹. This maturation process, and the maintenance of fertilising ability, is dependent on androgen². When testosterone levels are lowered by castration or hypophysectomy, sperm from the cauda epididymis have immature acrosomes (guinea pig)³ and lowered fertilising ability (rat)⁴. These effects are prevented by testosterone treatment. Since reduction of testosterone levels enhances the rate of

epididymal sperm transport^{4,5}, the incomplete maturation could result from spermatozoa spending insufficient time in critical regions of the epididymis, as well as from a decreased ability of the epididymis to promote sperm maturation. We demonstrate here that oestrogen and testosterone affect one aspect of sperm maturation in mice by altering the rate of epididymal sperm transport, and also affect fertility.

Male BC3F₁ hybrid mice were injected daily with either 10 μ g d⁻¹ oestradiol benzoate (OB), 80 μ g d⁻¹ testosterone propionate (TP), both hormones (OB+TP) or sesame oil alone. Administration of these hormones for up to 50 d produced negligible effects on the process of spermatogenesis in the testis (Table 1, Fig. 1). This observation is not inconsistent with other studies; the inhibition of spermatogenesis in mice by oestrogens is strain dependent⁷.

Table 1 Sperm content of testes, epididymides and vasa deferentia of hormone-treated mice (sperm heads per mouse $\times 10^{-6}$)

	Control	OB	OB+TP
Testes	51.6 $\pm$ 1.9	51.9 $\pm$ 1.7	47.2 $\pm$ 2.1
Capita epididymides	20.6 $\pm$ 0.8	1.4 $\pm$ 0.2*	7.2 $\pm$ 0.4*
Corpora epididymides	6.7 $\pm$ 0.8	2.0 $\pm$ 0.2*	3.8 $\pm$ 0.2*
Caudae epididymides	49.2 $\pm$ 1.4	10.8 $\pm$ 1.3*	27.8 $\pm$ 1.0*
Vasa deferentia†	3.6 $\pm$ 0.6	4.0 $\pm$ 0.6	2.9 $\pm$ 0.4

BC3F₁ male mice (C57BL $\times$ C3H δ from Cumberland View Farms, Clinton, Tennessee) were injected daily with hormone given intramuscularly in the leg in 0.05 ml of sesame oil for 39–50 d. Oestradiol benzoate (OB) was obtained as Oestroform (British Drug Houses); testosterone propionate (TP) as Perandren (Ciba). The sperm contents of these organs were determined by counting the number of sperm nuclei ('sperm heads') in sonicated tissue homogenates in a haemocytometer. The spermatid nucleus becomes resistant to sonication at late step 12, and remains sonication resistant throughout further maturation⁶. The sperm heads were prepared by homogenisation of the tissue in distilled water with a Polytron homogeniser, sonication and filtration through 10- μ m pore nylon cloth, to remove debris.

* Significantly different from controls at $P < 0.01$.

† In other experiments, OB significantly reduced the number of sperm in the vas, as well as in the epididymis, while having little effect on number of sperm in the testis.

In contrast, the sperm content of the epididymis was markedly reduced by OB treatment (Table 1). Treatment with OB+TP produced a smaller reduction. TP alone had little effect. Except for the decrease in sperm content and for a small, but significant, reduction in the height of the epithelial cells, the histological appearance of the epididymis was unchanged by OB treatment.

The reduced numbers of spermatozoa could result from lower testicular sperm production, resorption of spermatozoa in the epididymis, or an enhanced rate of sperm transport through the epididymis. Testicular sperm production was calculated by dividing the number of sonication-resistant sperm heads (Table 1) by the time span (5.9 d)⁶ from late step 12 through further maturation⁸. Similar values of 8.7×10^6 , 8.8×10^6 and 8.0×10^6 sperm d⁻¹ were obtained for control, OB and OB+TP treated mice, respectively.

Experiments with mice, unilaterally vasectomised for 6–10 d, demonstrated that resorption of sperm in the epididymis is negligible. The accumulations of spermatozoa in the ligated-side epididymides of these mice were 11×10^6 , 6.3×10^6 and 6.8×10^6 sperm d⁻¹ for the 2 epididymides in control, OB and OB+TP treated mice, respectively, closely matching the sperm production rates. In addition, the reduction in the total radioactivity of the sperm in the reproductive tract, which occurred between days 27 and 31 in OB treated mice as the radioactively labelled sperm passed out of the vas, was largely prevented by ligation of the vas.

The kinetics of the transport of spermatozoa from the testis through the epididymis and the vas deferens was studied by measuring the radioactivity in cell suspensions or in sperm nuclei from different segments of the reproductive tracts of mice,

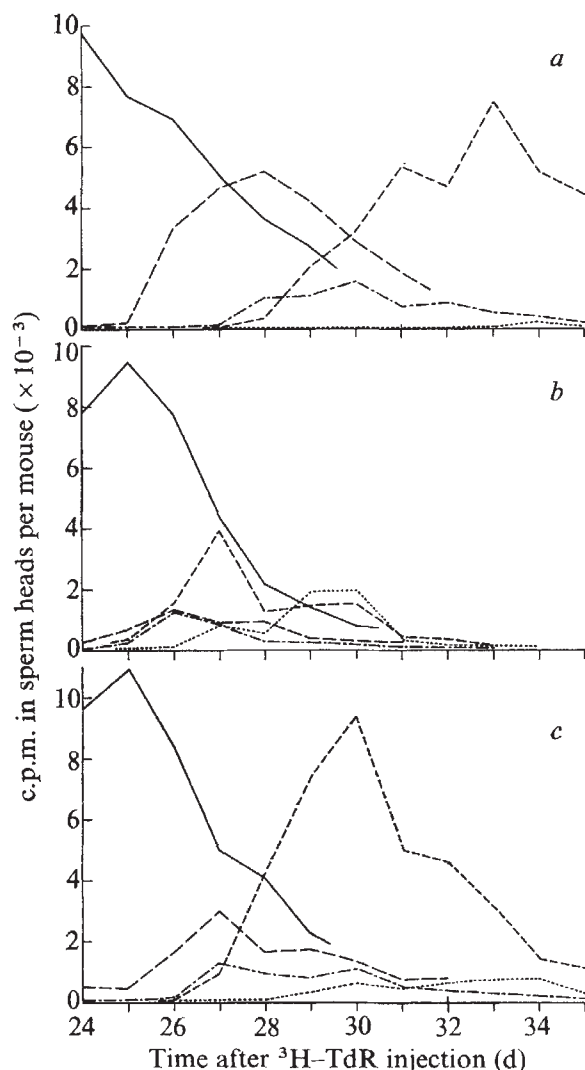


Fig. 1 Passage of radioactively labelled spermatozoa through the male reproductive tract of control (a), OB-treated (b) and OB+TP-treated (c) mice. Mice were given daily injections of the indicated hormone. On day 15 of hormone treatment, they were injected intraperitoneally with 30 μ Ci of 3 H-thymidine (TdR). Sperm heads, prepared from the testis (—), caput (---), corpus (---), cauda epididymis (---) and vas deferens (---) of mice killed on the indicated days, were collected by filtering onto Whatman GF/C filters. The radioactivity in the sperm heads was determined by liquid scintillation counting (30% efficiency). Data points (except for day 35) are averages of 3 samples, each prepared from one mouse. The average fractional standard errors in the counts between animals were 0.11, 0.15, 0.22, 0.24, 0.26 for the testis, caput, corpus, cauda epididymis and vas deferens respectively.

killed at daily intervals after injection of 3 H-TdR. The radioactivity in the testis was constant for 25 d after the administration of 3 H-TdR (data not shown), and then decreased similarly in all experimental groups (Fig. 1). In control and TP-treated mice, radioactive spermatozoa first entered the caput epididymis on day 26, the corpus on day 28, the cauda on day 29, and the vas not until day 34, or later. In OB and OB+TP-treated mice, however, radioactively labelled spermatozoa were present in the vas deferens on days 27 and 29 respectively.

The epididymal transit times calculated from these profiles clearly show a marked reduction with OB treatment and a less marked reduction with OB+TP treatment (Table 2). The reductions in epididymal sperm counts are a result of this increased transport rate, since transit times calculated from the sperm counts are nearly identical to those from radioactivity profiles.

Experiments were then performed to determine if one aspect

Table 2 Average epididymal transit times in hormone-treated mice (d)

Epididymal segment	Control	OB	OB+TP
Caput	2.3*(2.4)†	0.8 (0.2)	1.5(0.9)
Corpus	1.6 (0.7)	-0.1 (0.2)	0.5 (0.5)
Cauda	≥ 5.8 (5.6)	1.2 (1.2)	1.8 (3.6)

* Calculated from radioactivity profiles (Fig. 1). The average transit time for a given segment is the difference between the times at which labelled spermatozoa enter that segment and enter the next segment (start of rise of curves), or, equivalently, the difference between the times the spermatozoa leave the previous segment and leave that segment (maxima of curves). Unbiased estimates of these times were obtained by computer fitting the curves with exponential functions (manuscript in preparation). All values are averages of determinations from the times of entry and of leaving. For the control cauda epididymis, lower limits were chosen assuming labelled spermatozoa enter the vas after day 35.

† Calculated from sperm counts (Table 1). In the absence of sperm resorption, the same number of spermatozoa per day must pass through each point in the reproductive tract and that number equals the testicular sperm production rate (S). The number of spermatozoa present in any segment (n), equals the rate of entry of spermatozoa (S) times the average time a spermatozoon spends in that segment (t), which is the transit time. Therefore, $t = n/S$.

of sperm maturation, disulphide crosslinking of the sperm nuclear protein⁹, was affected by the hormonal treatment. Mouse sperm nuclei show progressive maturation during passage through the epididymis (Table 3). Sperm nuclei from each epididymal region of OB-treated mice were, however, less mature than those of control mice. This was not a direct result of OB treatment on sperm maturation, but rather a consequence of the enhanced rate of transport. Spermatozoa, blocked, by vasectomy, in the cauda epididymis and proximal region of the vas deferens of the OB-treated mice, showed increased disulphide crosslinking. This aspect of sperm maturation is time dependent, not necessarily dependent on the epididymal milieu, as expected from previous observations⁹.

The crucial aspect of sperm maturation is the development of fertilising ability. We tested mice, given hormonal treatment for 28 d before and during mating, by placing each male with two virgin females for two weeks. The males were then killed, and showed epididymal sperm counts, unaltered by mating. The females who did not bear litters were killed 14 d later, and checked for the presence of embryos. Nine out of nine control mice were fertile, but only two out of nine OB+TP-treated mice were fertile. All nine mice injected with OB were sterile. The fertility of the treated mice is significantly below that of controls ($P < 0.005$). Vaginal plugs were seen in females in all experi-

Table 3 Effect of hormonal treatment on sperm head maturation, measured by times (min) of incubation in SDS-DTT required for swelling

Source of sperm heads	Control	OB	OB+TP
Caput epididymis	12 $\pm$ 1	2	—
Cauda epididymis	21 $\pm$ 1	11 $\pm$ 1*	20 $\pm$ 0.3
Vas deferens	26 $\pm$ 0.5	19 $\pm$ 1†	—
Cauda-proximal vas from vasectomised mice			
Control side	26	12 $\pm$ 1	—
Vasectomised side	22	30 $\pm$ 3*	—

All mice were killed after 45–47 d of hormone treatment. Several mice were unilaterally vasectomised after 40 d of hormone treatment by ligating and cutting the vas deferens.

The extent of formation of disulphide linkages in the sperm nucleus was determined by the rate of swelling in 1% sodium dodecyl sulphate (SDS) and 2×10^{-3} M dithiothreitol (DTT) in 0.05 M sodium borate buffer (pH 9.0)⁹. Sonicated sperm heads were suspended in SDS-DTT and observed microscopically during incubation at room temperature (24 ± 1 °C) from 2 to 30 min. The level of swelling was scored on a 0–4 scale, and the time required to reach the level of 2 was determined by interpolation.

* Significantly different from controls at $P < 0.01$. In vasectomised mice, the statistical comparison was made between vasectomised and control sides.

† Significantly different from controls at $P < 0.05$.

mental groups. Further experiments are needed, however, to determine whether the infertility of OB-treated mice is a result of the immaturity of the sperm, or some other effect of the treatment.

The enhancement of epididymal sperm transport by oestrogen is a novel finding. The slowing of sperm transport by testosterone had been reported previously in the rat using ligation of the efferent ducts and/or vas deferens^{4,5}. Here, we confirm this phenomenon in mice without using ligation, which might perturb fluid and blood flow. The mechanisms responsible for sperm transport and its hormonal regulation are not known. Possibilities include peristaltic contraction of the epididymal tubules and action of the ciliated cells of the epididymal epithelium¹⁰. These mechanisms, however, seem to be inconsistent with our findings, since testosterone enhances epididymal contractions¹⁰ and the size of stereocilia¹¹. Our observation that the effect of OB is decreased by additional treatment with TP, indicates that OB may act to interfere with either the endogenous production or action of androgen. Oestrogen can reduce testosterone production by direct action on the Leydig cells, or by inhibiting the secretion of luteinising hormone¹². Morphological changes in the Leydig cells, which accompany depression of luteinising hormone levels¹³, were, however, not observed in OB-treated mice. Alternatively, oestrogen can act directly on the epididymis to reduce the effects of testosterone¹⁴, either by competitive binding to a testosterone receptor¹⁵, or by binding to an oestrogen receptor¹⁶ to produce a change opposite to that of androgen.

The results presented suggest the possibility of the regulation of male fertility with oestrogenic or antiandrogenic hormones. Such hormones could, without affecting testicular function, act on the epididymis to enhance the rate of sperm transport, resulting in immature spermatozoa in the ejaculate.

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- ¹ Bedford, J. M., in *Handbook of Physiology*, 5 (edit. by Greep, R. O., and Astwood, E. B.), 303-317 (American Physiology Society, Washington DC, 1975).
- ² Orgebin-Crist, M. C., Danzo, B. J., and Davies, J., in *Handbook of Physiology*, 5 (edit. by Greep, R. O., and Astwood, E. B.) 319-338 (American Physiology Society, Washington DC, 1975).
- ³ Blaquier, J. A., Cameo, M. S., and Burgos, M. H., *Endocrinology*, **90**, 839-842 (1972).
- ⁴ Dyson, A. L. M. B., and Orgebin-Crist, M. C., *Endocrinology*, **93**, 391-402 (1973).
- ⁵ Das, R. P., Roy, S., and Bandopadhyaya, G. P., *Contraception*, **8**, 471-484 (1973).
- ⁶ Meistrich, M. L., Reid, B. O., and Barcellona, W. J., *Expl Cell Res* (in the press).
- ⁷ Shimkin, M. B., Smith, S. J., Shimkin, P. M., and Andervont, H. B., *J. natn. Cancer Inst.*, **28**, 1219-1231 (1962).
- ⁸ Orgebin-Crist, M. C., *J. Reprod. Fert.*, **15**, 15-25 (1968).
- ⁹ Calvin, H. I., and Bedford, J. M., *J. Reprod. Fert. Suppl.*, **13**, 65-75 (1971).
- ¹⁰ Risley, R. L., in *Mechanisms Concerned with Conception* (edit. by Hartman, C. G.), 73-133 (MacMillan, New York, 1963).
- ¹¹ Orgebin-Crist, M. C., and Davies, J., *Cell Tissue Res.*, **148**, 183-201 (1974).
- ¹² Chowdhury, M., Tcholakian, R., and Steinberger, E., *J. Endocr.*, **60**, 375-376 (1974).
- ¹³ Huseby, R. A., Dominguez, O. V., and Samuels, L. T., *Rec. prog. Horm. Res.*, **17**, 1-51 (1961).
- ¹⁴ Lubicz-Nawrocki, C. M., *J. Endocr.*, **61**, 133-138 (1974).
- ¹⁵ Rennie, P., and Bruchovsky, N., *J. biol. Chem.*, **248**, 3288-3297 (1973).
- ¹⁶ van Beurden-Lamers, W. M. O., Brinkmann, S. E., Mulder, E., and van der Molen, H. J., *Biochem. J.*, **140**, 495-502 (1974).

Reduced deformability of erythrocyte membranes from patients with Duchenne muscular dystrophy

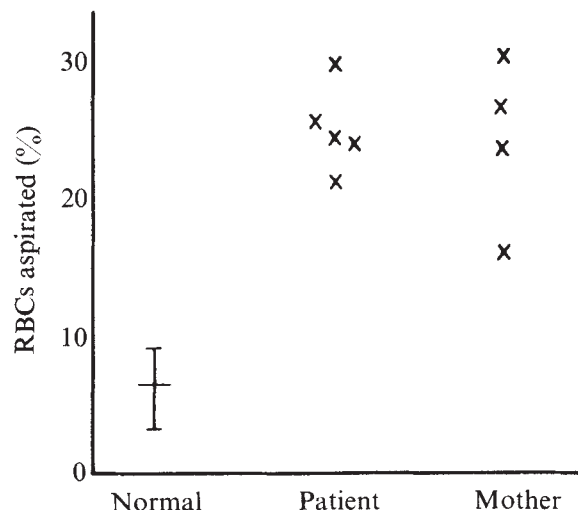
THE fundamental defect in Duchenne muscular dystrophy is obscure. Early investigators attributed the disorder to a primary defect of the skeletal muscle itself,¹ but alternative suggestions have implicated the motor neurone^{2,3} or the vascular supply⁴. Recent attention has been directed to the plasma membrane of muscle as well as of other tissues. Several authors have postulated a generalised membrane defect as the fundamental problem in this disorder⁵⁻⁷.

Matheson and Howland⁸, using a scanning electron microscope, examined the erythrocytes of patients with various forms of muscular dystrophy. When compared with control erythrocytes, the patients' cells exhibited striking alterations in surface contour. These changes were most prominent in cells derived from Duchenne patients. The obligate female carriers for Duchenne dystrophy also showed similar deformities of the erythrocyte surface. It is clear that these surface changes are not specific for the muscular dystrophies as such changes have also been noted clinically in association with severe liver disease, uraemia, splenectomy, haemoglobinopathies and β -lipoprotein deficiency. It was previously noted by Weed and Chailley⁹ that similar erythrocyte alterations could be induced in 'normal' erythrocytes by incubation in the presence of the divalent cation, calcium, or in solutions whose pH exceeded 8.5. The resulting cells, echinocytes, have also been produced following incubation of erythrocytes in the presence of a variety of chemical compounds including salicylates, barbiturates, and 2,4-dinitrophenol.

On the basis of observations of Matheson and Howland, we speculated that the altered surface contour of the Duchenne patients' erythrocytes may be reflected in abnormal membrane elasticity or deformability. To evaluate this property, we have used cell elastimetry modified from the original method of Mitchison and Swann⁹. This method has been used successfully in our laboratory in the study of polymorphonuclear leukocytes¹⁰ and was readapted readily to the study of erythrocytes.

Heparinised blood was obtained by venipuncture and the plasma removed by centrifugation. The cells were then washed three times in a balanced salt solution at pH 7.2. Micropipettes were pulled to an internal diameter of 1-2 μ m and attached to a sensitive source of negative pressure. With microscopic assistance, a micromanipulator was used to direct an erythrocyte to the pipette orifice. The amount of negative pressure

Fig. 1 Percentage of erythrocytes aspirated at negative pressures greater than 100 mmHg in preparations from controls, Duchenne dystrophy patients and obligate female carriers.



required to aspirate the erythrocytes into the pipette was determined. Measurements were not carried beyond negative pressures of 250 mmHg even though some cells were not aspirated at negative pressures exceeding 500 mmHg. The negative pressure required to aspirate an erythrocyte into the pipette could depend either on the fluidity of the cytoplasm or the plasma membrane. The latter seems more likely since red cell ghosts have been shown to exhibit the same deformability characteristics as intact erythrocytes¹¹.

Negative pressure measurements were carried out on 100 red cells from each control or patient and the percentage of cells aspirated at negative pressures in excess of 100 mmHg was computed.

We have used this technique on erythrocytes from five patients with Duchenne muscular dystrophy and four obligate female carriers. All diagnoses were substantiated by abnormal serum creatine phosphokinase (CPK) levels, muscle biopsy findings, and genetic analysis.

Of the Duchenne dystrophy erythrocytes, 25% were aspirated at negative pressures greater than 100 mmHg compared with only 6% of control erythrocytes (Fig. 1). It is interesting that erythrocytes from obligate female carriers exhibited a comparable reduction in deformability. Both observations are highly significant (Student's *t*-test).

The relevance of these observations may be considered from two perspectives. First, in terms of deformability, erythrocytes from Duchenne patients are easily distinguished from control cells as are those from the obligate female carriers. Since the measurement of serum CPK levels fails to delineate carriers consistently, the biological assay used in this study may offer an effective adjunct for carrier detection, and thus, for genetic counselling.

Second, and more fundamental, is the relevance of altered erythrocyte deformability in Duchenne dystrophy. To address this question, it is important to examine the mechanism of this deformability. Phagocytic cells, including PMNs have a recognisable microtubule-microfilament system distributed beneath the plasma membrane. Abundant data indicate that this system involves contractile proteins similar to, if not identical with actin and myosin. Recent evidence from our laboratory has indicated an association between membrane deformability and microtubule-microfilament function in PMNs¹². Although erythrocytes lack a microtubule system as such, proteins with a fibrillar appearance have been demonstrated on the cytoplasmic surface of the red cell membrane. These proteins are myosin- and actin-like and exhibit ATPase activity characterised as ouabain insensitive and divalent cation dependent^{13,14}. These properties are analogous to those of myo-fibrillar ATPase and to mitochondrial ATPase. This association of ATPase activity with fibrillar proteins on the inner surface of the red cell membrane provides a reasonable basis for understanding the process of erythrocyte membrane elasticity or deformability.

The observations of this study support the present notion of a generalised membrane defect in Duchenne dystrophy and suggest that further studies of the biochemical events associated with the dynamic process of red cell deformability may well explain the basic defect in this disorder.

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- ⁵ Rodan, S. B., Hintz, R. L., Sha'afi, R. J., and Rodan, G. A., *Nature*, **252**, 589 (1974).
- ⁶ Matheson, D. W., and Howland, J. L., *Science*, **184**, 165 (1974).
- ⁷ Roses, A. D., Herbstreith, M. H., and Appel, S. H., *Nature*, **254**, 350 (1975).
- ⁸ Weed, R. I., and Chailley, B., *Nouvelle Rev. Française d'Hémat.*, **12**, 775 (1972).
- ⁹ Mitchison, J. M., and Swann, M. M., *J. exp. Biol.*, **31**, 443 (1954).
- ¹⁰ Miller, M. E., *Sem. Hematol.*, **12**, 59 (1975).
- ¹¹ Weed, R. I., LaCelle, P. L., Merrill, E. W., *J. clin. Invest.*, **48**, 795 (1969).
- ¹² Miller, M. E., Myers, K. A., *Fedn Proc.*, **34**, 269 (1975).
- ¹³ Wins, P., and Schoffeniels, E., *Biochim. biophys. Acta*, **120**, 341 (1966).
- ¹⁴ Rosenthal, A. S., Kregenow, F. M., and Moses, H. L., *Biochim. biophys. Acta*, **196**, 254 (1970).

Enhanced *Trypanosoma musculi* infections in mice with concomitant malaria

A PHASE of immunodepression is characteristic of experimental malaria infections and, in mice, immune responses to antigens as diverse as sheep red blood cells¹, tetanus toxoid², oncogenic viruses³ and *Toxoplasma gondii*⁴ are diminished. From immunological and epidemiological viewpoints it is important to know what happens when animals are infected with another agent during this period of immunodepression. The agent must be carefully chosen; it must be able and likely to coexist with the malaria parasite *in vivo*, be easy to monitor and elicit a well defined immune response. *Trypanosoma musculi*, an avirulent murine trypanosome, fulfils these criteria. There is no cross

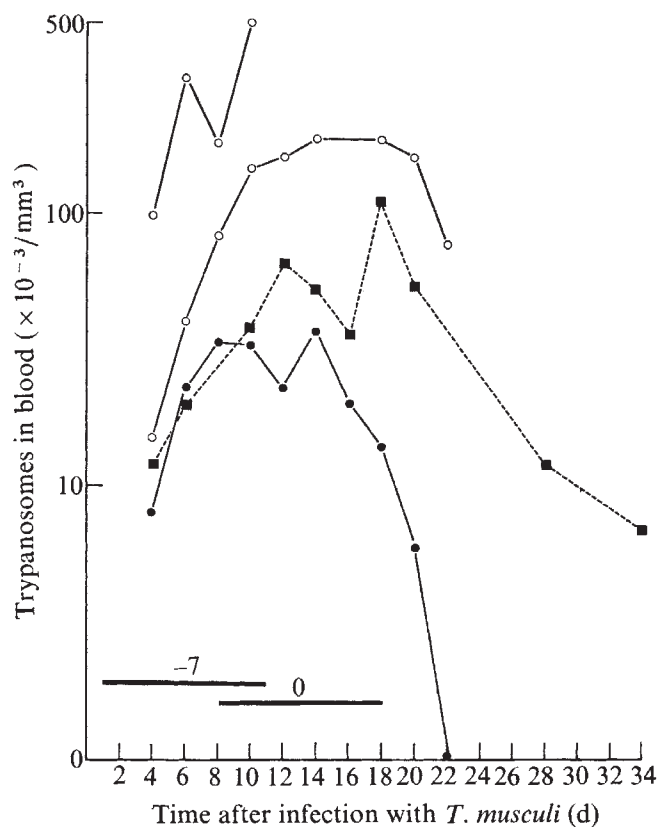


Fig. 1 Patterns of infection with *T. musculi* in female TO Swiss mice. Each point represents the mean of all control mice (●), mice infected with *P. yoelii* at the same time (■) and mice infected with *P. yoelii* 7 d earlier (○). This last group has been divided into subgroups; in one of these 5 mice died trypanosomiasis within 10 d whereas the remaining 13 survived for at least 21 d. The graph is incomplete because the death of some mice after day 22 and the fact that some mice had recovered from their infections while others still harboured parasites, would give a false impression of the nature of the infection during its final phase if the mean parasitaemias were plotted. This latter point also applies to the other experimental group. The solid blocks, -7 and 0, indicate the periods of immunodepression related to infection with *P. yoelii* 7 d before *T. musculi* and at the same time.

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- ¹ Walton, J. N., *Br. Med. J.*, **1**, 1271 (1964).
- ² Gallup, B., and Dubowitz, V., *Nature*, **243**, 287 (1973).
- ³ McComas, A. J., Sica, R. E. P., and Currie, S., *Nature*, **226**, 1263 (1970).
- ⁴ Hathaway, P. W., Engel, W. K., and Zellweger, H., *Archs Neurol.*, **22**, 365 (1970).

immunity between it and rodent malaria⁵, the immune response has been very carefully characterised⁶⁻⁸ and the progress of the infection can be monitored easily by taking minute quantities of blood. We have found that *T. musculi* infections are considerably enhanced in mice coincidentally infected with an avirulent malaria parasite, *Plasmodium yoelii* (*P. berghei yoelii*)⁹.

Batches of mice were infected intravenously with approximately eight *P. yoelii* parasites and challenged by the intravenous injection of about 1,000 *T. musculi* at the same time or 7 d later. The trypanosome infections were monitored and the results are shown in Fig. 1. Infections in 18 control mice resembled those described by Viens *et al.*⁸ and the mice overcame the infections within 22 d. In all the experimental animals, the infections were more intense and persisted for longer than in the controls. The greatest enhancement occurred in mice infected with *P. yoelii* 7 d earlier. In this group, 5 out of 18 mice died of trypanosomiasis within 10 d, one mouse harbouring over 5×10^5 trypanosomes per mm³ of blood just before it died. Of the remaining mice, a further four died on days 22–24 of a combination of malaria and trypanosomiasis, two died of recrudescence malaria 25 and 26 d after infection while still harbouring some trypanosomes and seven became negative between days 21 and 28. In 18 animals infected with *T. musculi* and *P. yoelii* at the same time the infections resembled those in control mice until day 10 but thereafter they continued to rise for a further 7–9 d before gradually declining until all the mice were negative on day 41. There were no fatalities in this group.

Immunodepression during *P. yoelii* infections coincides with the peak of parasitaemia and lasts for about 10 d (ref. 1). In mice infected with *P. yoelii* 7 d before *T. musculi* this period of immunodepression would have occurred during days 1–11 of the trypanosome infection, just when the control animals were beginning to overcome it. In mice infected with *P. yoelii* at the same time as *T. musculi* the period of immunodepression would have extended over days 8–18 and this is consistent with the observation that it is during this period that the infections in the experimental mice diverge from those in the controls.

Immunity to *T. musculi* has been shown to operate in two stages⁶⁻⁸. The first response after about 6 d inhibits reproduction and involves T-cell dependent ablastin and T-cell independent trypanocidal antibody. The second response brings about the elimination of the trypanosomes and is T-cell dependent. In T-cell deprived mice, *T. musculi* infections persist for the lifetime of the host⁶. This was not the case in our experiments and our results indicate that *P. yoelii* produces a defect in the first response. There is, as yet, no satisfactory explanation of immunodepression during malaria infections but a hypothesis which suggests that antibody producing cells, particularly those responsible for IgM, are affected is consistent both with our findings and with the well known fact that cells forming plaques to sheep red blood cells are considerably reduced during *P. yoelii* infections¹.

The epidemiological significance of immunodepression during malaria is considerable because the chances of transmission of a superimposed infection increase as the intensity and duration of that infection increase. There are also reciprocal effects between these parasites. In these experiments we have noted that *P. yoelii* infections are enhanced in some, but not all, mice infected with *T. musculi* with the result that mice may die unexpectedly from recrudescences of malaria or from the cumulative effect of the dual infection. We have also observed that the piroplasm *Babesia microti* enhances *T. musculi* infections and that both *P. yoelii* and *B. microti* modify *T. brucei* infections.

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- ¹ Salaman, M. H., Wedderburn, N., and Bruce-Chwatt, L. J., *J. gen. Microbiol.*, **59**, 383–391 (1969).
- ² Voller, A., Gall, D., and Manawadu, B. R., *Z. Tropenmed. Parasit.*, **23**, 152–155 (1972).
- ³ Wedderburn, N., *Lancet*, **ii**, 1114–1116 (1970).
- ⁴ Strickland, G. T., Voller, A., Pettit, L. E., and Fleck, D. G., *J. infect. Dis.*, **126**, 54–60 (1972).
- ⁵ Cox, F. E. G., *Parasitology*, **65**, 399–402 (1972).
- ⁶ Viens, P., Targett, G. A. T., Leuchars, E., and Davies, A. J. S., *Clin. exp. Immunol.*, **16**, 279–294 (1974).
- ⁷ Targett, G. A. T., and Viens, P., *Int. J. Parasit.*, **5**, 231–234 (1975).
- ⁸ Viens, P., Targett, G. A. T., and Lumsden, W. H. R., *Int. J. Parasit.*, **5**, 235–239 (1975).
- ⁹ Killick-Kendrick, R., *Parasitology*, **69**, 225–237 (1974).

T-cell activation in murine malaria

THE immune response of rodents to malaria is thymus dependent. *Plasmodium berghei* infections are more severe in neonatally thymectomised rats than in intact controls¹, whereas *P. yoelii* (*P. berghei yoelii*) infections which are self-resolving in normal CBA mice prove fatal in T-cell deprived CBA mice (Jayawardena *et al.*, unpublished) or congenitally athymic nude mice². Malarial infections are also known to depress immune responses to certain antigens. In the mouse, they reduce the antibody³ and splenic plaque forming cell⁴ response to sheep red blood cells (SRBCs), render animals more susceptible to lymphomagenic viruses⁵, and severe long term infections prolong xenogeneic skin graft survival time⁶. It has been suggested that such impaired immune reactivity reflects defective lymphocyte or macrophage function, but there has been no unequivocal evidence in support of these possibilities.

We have examined the T-cell mitotic response, the production of anti-parasite antibodies and parameters of T-cell function (the response to phytohaemagglutinin (PHA) and oxazolone) in non-fatal *P. yoelii* and fatal *P. berghei* infections in the CBA mouse, and present evidence suggesting that the development of protective immunity and the nonspecific immunodepression can be related to the T-cell response of the host.

We defined the T-cell response to infection in CBA/Lac-CBA/T₆T₆ mouse radiation chimaeras carrying a chromosomally distinguishable population of T cells. To prepare these radiation chimaeras CBA/Lac mice were thymectomised at 7 weeks of age, subjected to 850 rad X irradiation 10 d later, and reconstituted immediately with 5×10^6 femoral CBA/Lac bone marrow cells. Within 20 h of X irradiation, thymus grafts consisting of two thymus lobes from CBA/T₆T₆ neonates were implanted under the kidney capsule⁷. The grafts remained *in situ* for 30 d after which they were removed⁸. Twenty days later, these mice were infected with the 17X strain of *P. yoelii* or the Anka strain of *P. berghei* (all infections were initiated with an intraperitoneal inoculum of 1×10^5 parasitised RBCs). At various times after infection the spleens were removed and processed for cytological analysis⁹ and the proportion of dividing cells, known by their chromosome marker to be of thymus graft origin, was determined. (A minimum of 100 dividing cells were scored at each stage of infection.)

The patterns of parasitaemia and the T cell mitotic response in the spleens of infected mice are shown in Fig 1. In mice infected with *P. yoelii*, parasites were detected in the peripheral blood within 2 d and peak parasitaemias were recorded on day 10, after which the infection regressed, all mice being aparasitaemic by day 18. Within 4 d of infection (when the percentage parasitaemia was

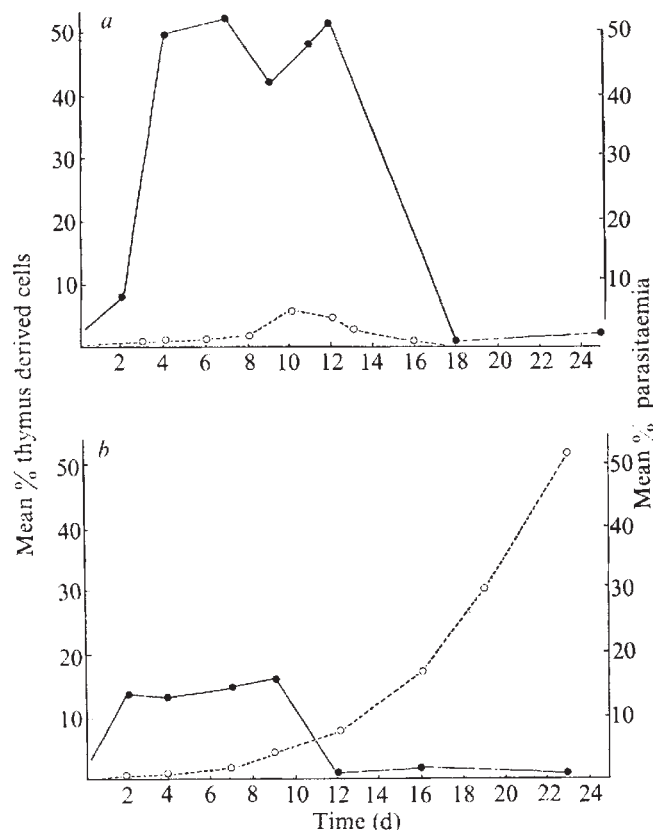


Fig. 1 Mean percentage of thymus graft derived cells (●) dividing in the spleen and mean percentage parasitaemias (○) in CBA/Lac-CBA/T₆T₆ mouse radiation chimaeras infected with 1×10^5 *P. yoelii* (a) or *P. berghei* (b) parasitised RBCs.

<1%), *P. yoelii* infected mice showed massive T-cell activation—53.1% of the dividing cells in the spleen being of certain thymic origin. This level of T-cell activation was maintained until day 13. Between days 13 and 18, during which time control of the infection seemed to have been achieved, as judged by the clearance of parasites from the peripheral blood, T-cell mitoses became much less frequent.

During the first 10 d of infection with *P. berghei*, parasitaemias were similar to those observed in mice infected with *P. yoelii*. Thereafter the parasitaemias increased steadily and proved fatal by day 24. The amount of T-cell activity elicited during a fatal *P. berghei* infection was

Table 1 Fluorescent antibody titres in CBA mice infected with *P. yoelii* or *P. berghei*. Each determination was carried out on serum pooled from five mice using the indirect fluorescent antibody method for malaria parasites as described by Voller¹⁰

	Day of infection	Total Ig*	IgG ₁ †	IgG ₂	IgM
<i>P. yoelii</i>	0	—	—	—	—
	7	32	—	32	256
	14	1,024	512	2,048	128
	21	1,024	128	512	256
	28	1,024	256	1,024	256
	35	1,024	256	512	128
<i>P. berghei</i>	0	—	—	—	—
	5	—	—	—	32
	9	32	—	32	128
	15	32	32	64	256
	18	64	128	128	64
	24	64	128	256	64

*Lyophilised swine anti-mouse globulin conjugated with fluorescein isothiocyanate (FITC) was purchased from Nordic Pharmaceuticals.

†Goat anti-mouse IgG₁, IgG₂ and IgM sera were obtained from Nutritional Biochemical Corporation, Cleveland, and were conjugated to FITC¹¹ isomere 1 (Nordic Pharmaceuticals).

proportionately much less than that observed in a resolving *P. yoelii* infection. Although by day 2 13.7% of the cells in mitosis were T cells (compared with 7.4% in *P. yoelii*), there was little change in the level of T-cell activation over the next 7 d and, from day 9 onwards, the response seemed to fail completely in spite of the continuously increasing parasite burden.

The precise functional significance of the T-cell mitosis observed in these infections is uncertain, but such mitosis could be indicative of the development of cytotoxic, helper,

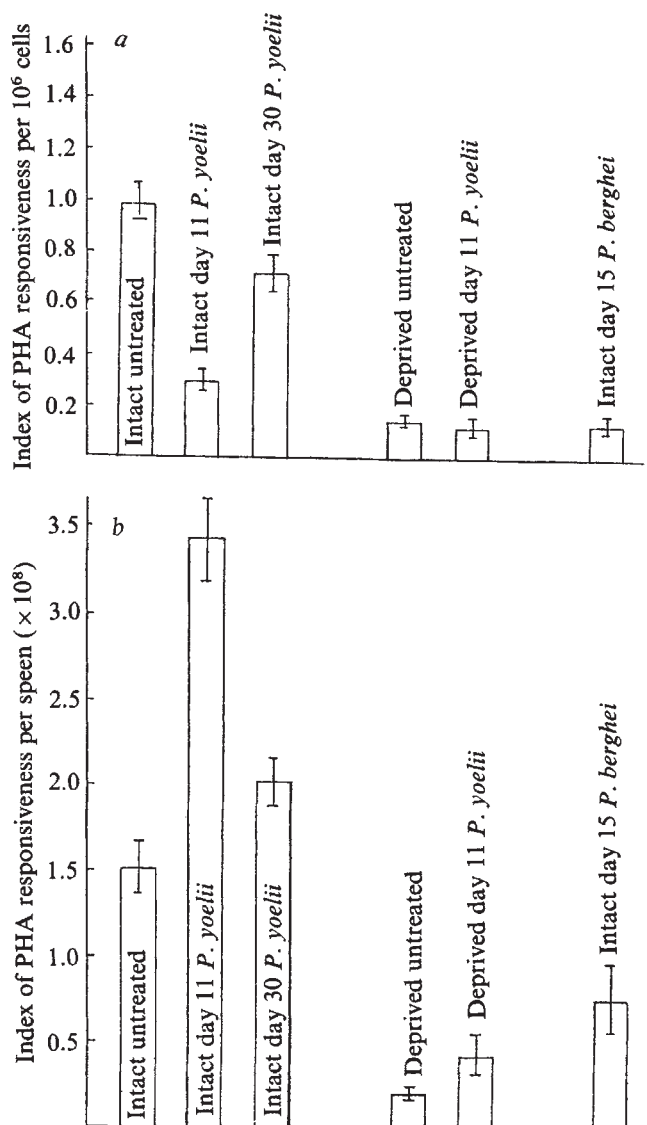
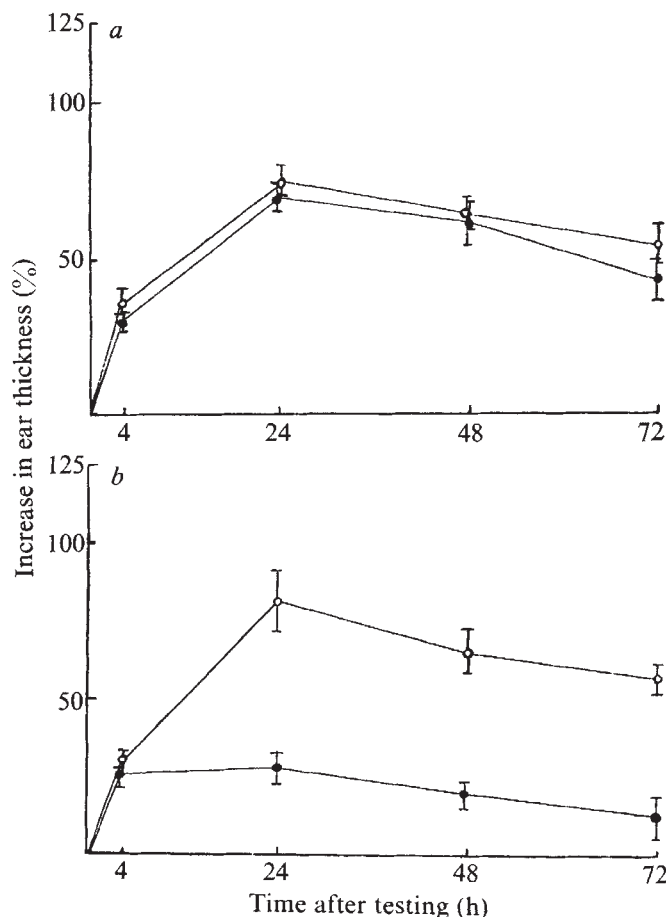


Fig. 2 The index of PHA responsiveness per 10^6 spleen cells (a) and the index of PHA responsiveness per spleen ± 1 s.e. (b) in normal and *P. yoelii* or *P. berghei* infected CBA mice. (Uninfected and *P. yoelii* infected T-cell deprived mice were included as additional controls.) Each group consisted of five mice. The method of quantitation used was that of Doenhoff¹³ and is based on the cytological analysis of cells derived from CBA/Lac and CBA/T₆T₆ mice. (Cells of the T₆T₆ strain carry a pair of minute chromosomes by means of which they can be distinguished at metaphase.) A known number of cells from untreated CBA/T₆T₆ mice was mixed with an equal number of cells from CBA/Lac mice whose response to PHA was being determined. The mixture of the two cell types was cultured with PHA (Burroughs Wellcome) for 3 d, when, following metaphase arrest with colcemid, the cells were subjected to cytological analysis⁹. The ratio of the dividing cells from the two sources (Lac:T₆T₆) gave the relative concentration of PHA responsive cells, and this was designated the index of responsiveness per 10^6 cells. (A culture consisted of 1×10^6 cells.) To estimate the degree of responsiveness per spleen, the index per 10^6 cells was multiplied by the total number of nucleated cells in the spleen from which the cultured cells were derived.

or some other T-cell-dependent effector activity such as lymphokine production. In *P. yoelii* infected mice, considerable T-cell activation occurred early in the infection at a time when only very low levels of antibody could be detected. (Fluorescent antibody levels are recorded in Table 1.) Once the parasitaemia and T-cell activity began to decline, peak levels of antibody were recorded and considerably elevated levels of these antibodies were maintained long after the T-cell response had died down. It must be pointed out, however, that this temporal relationship between antibody production and the T-cell response could be due to excess circulating antibody being present only after regression of the blood parasitaemia. *P. berghei* infected mice produced significantly lower levels of antibody particularly in the IgG₁ and IgG₂ subclasses.

In a parallel series of experiments we examined two correlates of T-cell function—the response of spleen cells to the T-cell mitogen PHA¹² (Fig. 2), and the response of infected animals to the skin sensitising agent oxazolone (Fig. 3). On day 11 of infection (the time of peak parasitaemia), *P. yoelii* infected mice showed a reduction in the proportion of PHA-responsive cells in the spleen. By this time, however, spleen cell numbers had increased from 1.53×10^8 on day 0 to 12.22×10^8 . Thus, when the response was computed on a per spleen basis there seemed to be no absolute deficiency in PHA responsiveness—the response per spleen in the infected group being 3.42×10^8 compared with 1.51×10^8 in the controls. Thirty days after infection,

Fig. 3 Mean percentage increase in ear thickness ± 1 s.e. in uninfected ($\circ$) and *P. yoelii* (a) or *P. berghei* (b) infected ($\bullet$) CBA mice sensitised with oxazolone (five mice per group). Mice were sensitised (on day 8 of infection) by applying 0.1 ml of a 3% solution of oxazolone in alcohol to the skin of the abdomen and were tested 6 d after sensitisation by applying one drop of a 1% solution of oxazolone in olive oil on to both sides of the right and left ears¹⁴. Ear thickness was measured at 4, 24, 48 and 72 h after application of the test solution.



both the proportion and total number of PHA-responsive cells in the spleen showed no significant difference from control values. In contrast, *P. berghei* infected mice, when assayed on day 15 of infection, showed a severe reduction in the proportion of responsive cells comparable with that found in T-cell deprived mice and, even when calculated on a per spleen basis, the response was still significantly depressed. The ability to respond to oxazolone was also seriously impaired in *P. berghei* infected mice, whereas the response of *P. yoelii* infected mice, at the time tested, seemed normal.

It can thus be seen that these two parasites exert very different effects on the T-cell pool; *P. yoelii* induces massive T-cell activation, the development of protective immunity and the responses to PHA and oxazolone seem normal. *P. berghei*, on the other hand, elicits limited T-cell activity—there being a failure of the T-cell response to the parasite and a generalised failure of T-cell function. Although transient depression of immune responsiveness to antigens such as SRBC, HGG (heat aggregated human γ globulin)⁴ and tetanus toxoid¹⁵ have been shown to occur *P. yoelii* infections—this is not necessarily due to suppression of T-cell function. Architectural changes in the spleen and consequent changes in cell relationships, dilution of responsive cells by non-responsive elements or some form of pre-emption¹⁶ may be relevant to this temporary loss of immune responsiveness and may also contribute to the immuno-suppression in *P. berghei* infections.

Although it is not possible on the basis of the results presented here to establish definitely a causal relationship between T-cell activity and the development of immunity or the nonspecific suppression of T-cell function, these results suggest strongly a crucial role for T-cell activation and T-cell suppression in the pathogenesis of malaria.

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- 1 Brown, I. N., Allison, A. C., and Taylor, R. B., *Nature*, **219**, 292–293 (1968).
- 2 Clarke, I. A., and Allison, A. C., *Nature*, **252**, 328–329 (1974).
- 3 Salaman, M. H., Wedderburn, M., and Bruce-Chwatt, L. J., *J. gen. Microbiol.*, **59**, 383–391 (1969).
- 4 Greenwood, B. M., Playfair, J. H. L., and Torrigiani, G., *Clin. exp. Immunol.*, **8**, 467–478 (1971).
- 5 Wedderburn, N., *Lancet*, **ii**, 1114–1116 (1970).
- 6 Sengers, R. C. A., Jerusalem, C. R., Doesburg, W. H., *Exp. Parasitol.*, **30**, 41–53 (1971).
- 7 Leuchars, E., Cross, A. M., and Dukor, P., *Transplantation*, **3**, 28–38 (1965).
- 8 Doenhoff, M. J., and Davies, A. J. S., *Cell. Immun.*, **2**, 82–91 (1971).
- 9 Ford, C. E., in *Tissue Grafting and Radiation* (edit. by Micklem, H. S., and Loutit, J. F.), 197–206 (Academic, New York and London, 1966).
- 10 Voller, A., *Bull. Wld Hlth Org.*, **30**, 343–354 (1964).
- 11 Thompson, S. W., *Selected Histochemical and Histopathological Methods* (Thomas, Springfield, Illinois, 1966).
- 12 Doenhoff, M. J., Davies, A. J. S., Leuchars, E., and Wallis, V. J., *Proc. R. Soc.*, **B176**, 69–85 (1970).
- 13 Doenhoff, M. J., *Clin. exp. Immunol.*, **8**, 603–615 (1971).
- 14 Asherson, G. L., and Ptak, W., *Immunology*, **15**, 405–416 (1968).
- 15 Voller, A., Gall, D., and Manawadu, B. R., *Z. Tropenmed. Parasitol.*, **23**, 152–155 (1972).
- 16 O'Toole, C. M., and Davies, A. J. S., *Nature*, **230**, 187–188 (1971).

Genetics of natural resistance to herpesvirus infections in mice

INHERITED resistance to virus infections has been demonstrated in several murine systems¹⁻⁶. Susceptibility to mouse hepatitis virus³ and resistance to arbovirus⁷ infections in mice are dominant traits associated with the capacity of macrophages from the susceptible strains to replicate virus. Resistance to Friend leukaemia virus- and Gross leukaemia virus-induced leukaemogenesis is determined by at least two genes, one closely linked to the immune response genes of the mouse⁸. Conversely, susceptibility to lymphocytic choriomeningitis (LCM) virus infections in mice has been associated with a gene closely linked to the immune response genes⁹. The latter seems to be associated with the cell-mediated immune response to LCM which causes tissue damage and leads to death. Cell-mediated immunity seems to be important in resistance against herpes simplex virus (HSV) infections¹⁰⁻¹². Analysis of the aspects of cell-mediated immunity which might be involved in resistance in man is complicated by the uncontrollable factors associated with activation of latent herpesvirus infections. I have developed a mouse model which has shown that at least three genes are responsible for resistance to HSV. Although preliminary evidence suggests that resistance is immunologically mediated, resistance genes do not seem to be closely linked to the histocompatibility region of the mouse.

We used inbred strains of mice with high and low resistance. Since resistance to viral leukaemogenesis was demonstrated with an unusually virulent strain of virus¹³ (Gross passage A¹⁴), we used an HSV-1 strain (2931) which is highly virulent in BALB/c mice. The strain was isolated from a human labial lesion and passaged ten times in Vero cells. The virus was then cloned to produce a homogeneous virus population, and thus a homogeneous response from the various mouse strains. Cloned virus was typed by the neutralising antibody test with antisera provided by Dr Donald Armstrong, Memorial Sloan-Kettering Cancer Center. A large pool was prepared by infecting Vero cells at a multiplicity of 1 plaque-forming-unit (PFU) per cell and incubating the cells for 24 h at 37 °C. Cells and debris were removed from the supernatant by centrifugation at 150g and the virus was stored at -80 °C in 1-ml samples. This preparation had a titre of 1×10^8 PFU ml⁻¹ when titred at the beginning and end of the experiments. The same pool was used throughout.

Eleven strains of inbred mice were obtained from Jackson Laboratories and tested for their capacity to resist fatal HSV-1 infections. Adult (3-4-month-old) male mice were inoculated

intraperitoneally with the stock pool of HSV-1. Six to twelve mice were inoculated with 0.1 ml containing $1-10^8$ PFU per inoculum. Mice were observed for 21 d and an LD₅₀ was determined for each strain by the Spearman-Kärber method¹⁵ (Table 1). Mice usually showed signs of encephalitis and died 5-10 d after inoculation. AKR, A, BALB/c, and C57BL/6 mice were provided by Dr E. A. Boyse of Memorial Sloan-Kettering Cancer Center to confirm that the resistance observed in the mice from Jackson Laboratories could be observed in the same strains from other sources. LD₅₀s were essentially the same and have been incorporated into Table 1. The eleven strains seem to fall into three categories: resistant, moderately susceptible and very susceptible. Most resistant mice survived inocula of 10^6 PFU of HSV-1, while LD₅₀s for moderately susceptible mice are in the range 200-1,000 PFU per inoculum. The LD₅₀s for very susceptible mice are in the range 10-70 PFU per mouse. Susceptible mice which survived the lower doses of virus were reinoculated 4-8 weeks later with 10^6 PFU of HSV-1. All survived reinoculation, indicating that they had developed resistance.

To determine whether this pattern of resistance and susceptibility was the same for other HSV-1 strains, similar experiments were carried out with three further wild strains of HSV-1 as well as with HSV-1 strains obtained from Dr Fred Rapp (through Michael Bernhardt), Dr Barry Bloom and from the American Type Culture Collection (ATCC). The MacIntyre strain (VR₃) is the prototype HSV-1 strain held by the ATCC and has been used in studies of resistance by Nahmias *et al.*¹⁰ and Stevens and Cook¹⁵. All strains of HSV-1 have the same relative virulence in C57BL/6, BALB/c and A/J mice, as observed for HSV-1 (strain 2931) (Table 2). The exception was strain HEFM¹⁶ which seems to have lost its virulence for mice. HSV-1 (strain 2931) seemed to be more virulent than the other strains and continues to be used for this study.

Table 2 Virulence of HSV-1 strains

Strain (inoculum)	% killed by HSV-1		
	A/J	BALB/c	C57BL/6
2931 (1×10^4 PFU)	100	100	0
A.P. (2.7×10^4 PFU)	100	100	0
1895 (1×10^5 PFU)	100	60	0
MacIntyre (5×10^4 PFU)	100	60	0
Patton (1.67×10^3 PFU)	100	20	0
M.D. (1.3×10^4 PFU)	60	20	0
HEFM (2.2×10^6 PFU)	0	0	0

Five mice of each prototype strain were inoculated intraperitoneally with 0.1 ml of the seven HSV-1 strains listed. The amounts of virus inoculated is in parentheses. All mice were observed for 21 d and the percentage killed by each virus strain was calculated.

To determine whether resistance is dominant, crosses were produced between resistant and very susceptible mice (C57BL/6 $\times$ A/J)_{F₁} and between resistant and moderately susceptible mice (C57BL/6 $\times$ BALB/c)_{F₁}. Twenty of each of these F₁ animals were inoculated with 10^6 PFU of HSV-1 (Table 3). Ninety per cent of the (C57BL/6 $\times$ A/J)_{F₁} mice and 100% of the (C57BL/6 $\times$ BALB/c)_{F₁} mice survived, indicating that resistance was dominant.

Further crosses were made to determine the number of genes involved in resistance (Table 3). (C57BL/6 $\times$ A/J)_{F₁} mice were backcrossed to A/J strain mice. Forty-five adult progeny were inoculated with 10^6 PFU of HSV-1 and 34 (75.5%) of these died. If one dominant gene were involved, assuming parental homozygosity, 50% of the mice would have been susceptible. These data indicate that at least two genes are involved in resistance. (C57BL/6 $\times$ BALB/c)_{F₁} mice were backcrossed to BALB/c mice and 37 of these were inoculated with 10^6 PFU of virus. Only three (8%) of these died. This makes the genetic evaluation more difficult and may indicate that four or more genes are involved in resistance. Specific interaction between genes would also make an evaluation of this type difficult.

Resistance to some murine virus infections is determined by

Table 1 Resistance of inbred mouse strains to HSV-1

Mouse strain	H ₂	LD ₅₀ HSV-1 (P.F.U.)
AKR (12)*	k	$10^{1.0}$
SWR (6)	q	$10^{1.0}$
A (12)	a	$10^{1.33}$
DBA (6)	d	$10^{1.83}$
CBA (6)	k	$10^{2.34}$
BALB/c (12)	d	$10^{2.34}$
C3H/He (10)	k	$10^{3.0+}$
C57BR (6)	k	$10^{3.0+}$
C57BL/6 (12)	b	$> 10^6$
C57BL/Ks (6)	d	$> 10^6$
C57BL/10 (20)	b	$> 10^6$

Mice were inoculated intraperitoneally with 0.1 ml of tenfold dilutions of stock HSV-1 pool and observed for 21 d. Mice died with signs of encephalitis usually 5-10 d after inoculation. LD₅₀ was determined by the Spearman-Kärber method¹⁵.

*No. test per dilution.

†Titrations not clear cut. Two C3H and four C57BR mice survived at lower dilutions. Others died as if to yield the LD₅₀ noted. In a second experiment, 20 of each strain were inoculated with 10^6 PFU. Sixty per cent of the C3H/He and none of the C57BR mice died.

Table 3 Resistance of crosses inoculated with 10^6 PFU HSV-1

C57BL/6 (40*)	90% live
BALB/c (20)	0% live
A (20)	0% live
(C57BL/6 × A) _{F1} (20)	90% live
(C57BL/6 × BALB/c) _{F1} (20)	100% live
(C57BL/6 × A) _{F1} backcrossed to A (45)	25% live
(C57BL/6 × BALB/c) _{F1} backcrossed to BALB/c (37)	92% live

Mice were inoculated intraperitoneally with 0.1 ml HSV-1 containing 10^6 PFU. Percentage of mice surviving 21 d was calculated.

*No. of mice per test.

genes linked to the major histocompatibility complex of the mouse. Our preliminary results indicate that resistance to HSV-1 is not associated with a particular histocompatibility (H-2) allele. Resistant mouse strains were of H-2^b and H-2^d types. One susceptible and one moderately susceptible strain was also found to be of the H-2^d type. H-2H and H-2I mice were obtained from Dr Boyse to study this question further. These mice are crosses between C57BL/6 (resistant) and A/J strain (very susceptible) mice in which there is a crossover between the K and D ends of the H-2 region. The H-2H strain carries the K (I_r) region of the H-2I (A/J strain) and the D region of the H-2^b (C57BL/6), and the H-2I carries the reciprocal H-2 crossover. If the K region of the A/J strain is associated with susceptibility to HSV-1, as has been shown for susceptibility to viral leukaemogenesis, the H-2H should be susceptible. They were resistant (86%) to 10^3 PFU of HSV-1. Thus either the H-2 region of the A/J strain mice is not associated with susceptibility to HSV-1 or it is being masked by another resistance gene.

We also used congenic mice obtained from Dr Boyse. Eleven C57BL/6 mice into which the H-2^k allele of the AKR strain had been introduced (B6/H-2^k) and five AKR mice into which the H-2^b allele of C57BL/6 had been introduced (AKR/H-2^b) were inoculated with 10^3 PFU of HSV-1. The B6/H-2^k mice were resistant while the AKR/H-2^b mice were susceptible, indicating that the H-2^k allele did not confer susceptibility on C57BL/6 mice and the H-2^b allele did not confer resistance on AKR mice. These studies must be confirmed with more mice and a broader range of inocula. More definitive evidence for an association of H-2 allele with resistance will come from H-2 typing of resistant and susceptible backcross progeny.

Although genes governing resistance to HSV-1 do not seem to be closely linked to the H-2 complex, and thus the immune response genes of the mouse, preliminary evidence indicates that resistance is not a property of the susceptibility of structural cells and is probably mediated immunologically. Embryo fibroblast cultures were prepared from A/J, BALB/c and C57BL/6 mice and inoculated with HSV-1. Virus replicated to the same titre in each monolayer, indicating that resistance was not mediated by the inability of HSV-1 to replicate in structural cells of resistant mice. Preliminary lymphocyte transformation studies with splenic lymphocytes of mice inoculated 5–6 d earlier with HSV-1 indicate that resistant and moderately susceptible mice were capable of a vigorous response whereas very susceptible mice did not respond above control level (C.L. and R. O'Reilly, unpublished). *F*₁ crosses between resistant and very susceptible mice were also capable of a strong lymphocyte transformation response to HSV-1, indicating that this characteristic is inherited as a dominant trait.

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- Sabin, A. B., *Proc. natn. Acad. Sci. U.S.A.*, **38**, 540 (1952).
- Schell, K., *Aust. J. exp. Biol. Med. Sci.*, **38**, 289 (1960).
- Bang, F. B., and Warwick, A., *Proc. natn. Acad. Sci. U.S.A.*, **46**, 1065 (1960).
- Goodman, G. T., and Koprowski, H., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 160 (1962).
- Lindenman, J., *Proc. Soc. exp. Biol. Med.*, **116**, 506 (1964).
- Chang, S. S., and Hildemann, W. H., *J. natn. Cancer Inst.*, **33**, 303 (1964).
- Groschel, D., and Koprowski, H., *Arch. Ges. Virusforsch.*, **17**, 379 (1965).
- Lilly, F., and Pincus, T., *Adv. Cancer Res.*, **17**, 231 (1973).
- Oldstone, M. B. A., Dixon, F. J., Mitchell, G. F., and McDevitt, H. O., *J. exp. Med.*, **137**, 1201 (1973).
- Nahmias, A. J., Hirsch, M. S., Kramer, J. H., and Murphy, F. A., *Proc. Soc. exp. Biol. Med.*, **132**, 696 (1969).
- Zisman, B., Hirsch, M. S., and Allison, C. A., *J. Immun.*, **104**, 1155 (1970).
- Mori, R., Tasaki, T., Kimura, G., and Takeya, K., *Arch. Ges. Virusforsch.*, **21**, 459 (1967).
- Lilly, F., Boyse, E. A., and Old, L. J., *Lancet*, **ii**, 1207 (1964).
- Gross, L., *Proc. Soc. exp. Biol. Med.*, **94**, 767 (1957).
- Dougherty, R. M., in *Techniques in Experimental Virology* (edit. by Harris, R. J. C.), (Academic, New York, 1965).
- Stevens, J. G., and Cook, M. L., *J. exp. Med.*, **133**, 19 (1971).
- Rager-Zisman, B., and Bloom, B. R., *Nature*, **251**, 542 (1974).

Prevention of rabies by vitamin C

MURATA¹ reported on the virucidal activity of vitamin C and stated that vitamin C prevents various virus diseases. Clinical trials by F. Morishige (unpublished) showed that the incidence of transfusion hepatitis was decreased drastically when vitamin C was injected (3–7 g d⁻¹) for 1–2 weeks after blood transfusion, and Murata has pointed out that vitamin C is effective in the therapy of measles and mumps¹. I have now found that it also helps to prevent rabies.

Guinea pigs, fixed rabies virus and vitamin C (Lek, Ljubljana) were used in the experiments. Five groups of ten test animals and ten control animals were inoculated intramuscularly with 1 ml of a 10% emulsion of rabbit brain containing LD₅₀–LD₉₀ of the infectious fixed rabies virus. Treatment of the test animals was initiated 6 h after inoculation, and vitamin C was injected intramuscularly twice a day for 7 d; each dose contained 100 mg per kg body weight. Control animals were injected with saline. The animals were observed for 14 d. Most of those that succumbed to the inoculation became paralysed on the sixth or seventh day. Some became paralysed on the fifth day, and a few others not until the eighth or tenth day.

The frequencies of deaths from rabies in the five groups treated with vitamin C were: 5/10, 2/9, 6/9, 1/10, 3/10. Of the control animals, however, 9/10, 6/10, 9/10, 6/10 and 5/10 died (Table 1). Thus 17/48 (35.42%) of the treated animals and 35/50 (70%) of the controls died of rabies. The χ^2 value for these frequencies is 11.107, so that the difference between the treated and control groups is statistically significant ($P > 0.01$). My results therefore justify the conclusion that vitamin C is effective in rabies prevention. It had no therapeutic effect, however, for in animals which developed paralysis, continued treatment was always ineffective.

Table 1 Frequencies of deaths in guinea pigs inoculated with fixed rabies virus and treated with vitamin C and in the control animals.

Treatment	Deaths	Survivors	Σf
With vitamin C	17	31	48
Controls	35	15	50
Σf	52	46	98

In preliminary experiments, not reported here, lower doses of vitamin C (25 and 50 mg kg⁻¹) were tried in a small group of guinea pigs. These doses were less effective than the high dose used in the reported experiments. To establish the optimal dose of vitamin C, however, exact dose–response experiments will have to be done on sufficiently large groups of animals.

To exclude the possibility that rabbit brain itself contributed in any way towards paralysis and mortality of guinea pigs, another group of 30 guinea pigs was inoculated with 1 ml of a

10% emulsion of rabbit brain which was free of virus. All animals survived an observation period of 14 d without any signs of disease.

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¹ Murata, A., *Abstr. 1st Intersectoral Congr. Int. Ass. Microbiol. Soc., Tokyo* (edit. by Science Council of Japan, 1974).

Increased insulin binding capacity of liver membranes from diabetic Chinese hamsters

THE first evidence for an abnormality in insulin receptor capacity in pathological states was provided by Kahn *et al.*¹, who found that liver plasma membranes from the obese hyperglycaemic mouse bound only a fraction of insulin compared with membranes from lean littermates. A similar observation was made with lymphocytes from obese insulin-resistant diabetic patients². In lymphocyte cultures the insulin concentration in the medium seemed to determine the binding capacity of the cells³. These results suggested an inverse relationship between the number of receptors and the surrounding concentration of insulin, regardless of the tissue sensitivity to the hormone. We have now found greater than normal binding capacity in the diabetic Chinese hamster, which has genetic insulin deficiency, in many ways resembling juvenile diabetes⁴.

Table 1 Characteristics of control and diabetic groups and the respective plasma membrane preparations (10 animals in each group)

	Control	Diabetic
Body weight (g)	36.9 ± 0.3	34.8 ± 0.8
Liver weight (mg)	1.45 ± 0.05	1.71 ± 0.04
Blood sugar (mg per 100 ml)	130 ± 6	468 ± 59
5'-Nucleotidase (μmol mg ⁻¹ × min)	0.36	0.33
Mg ²⁺ -ATPase (nmol mg ⁻¹ × min)	179.2	168.8
Recovery of membrane protein (mg per g wet weight)	1.03	0.94

5'-Nucleotidase was measured as recommended by Solyom *et al.*⁶ Mg²⁺-ATPase was assayed by measuring the release of ³²P-orthophosphate from γ-³²P-ATP (ref. 7).

Plasma membranes were prepared by the method of Neville⁵ from diabetic Chinese hamsters aged 4–5 months and from control animals of comparable age and weight (Table 1). ¹²⁵I-insulin binding was assayed after incubation for 20 min at 25 °C; membrane-bound hormone was separated from free hormone by centrifugation through silicon oil (W.K., R.R., A. Zynamon and K.D.H., in preparation). Figure 1 is a Scatchard plot from a representative experiment. As previously observed in rats, the points fit two curves corresponding to high affinity–low capacity (site I) and low affinity–high capacity (site II) binding sites⁸. The constants derived from this figure are—for site I: control, 4.2 × 10⁻¹³, diabetic, 9.0 × 10⁻¹³ mol per mg protein; for site II: control, 1.9 × 10⁻¹², diabetic, 4.0 × 10⁻¹² mol per mg protein. The corresponding affinity constants are approximately 10⁻⁹ for site I, and 10⁻⁸ mol l⁻¹ for site II, in both groups. Recovery of membrane protein and enzyme markers was similar for both groups (Table 1). Figure 1 shows that the binding capacity for both classes of binding sites (intercept at abscissa) per mg protein is considerably higher in the diabetic animals, whereas there is no apparent change in affinity (represented by the slope). Although other interpretations of nonlinear Scatchard plots, such as negative cooperativity⁹, polymerisation of the hormone¹⁰ or

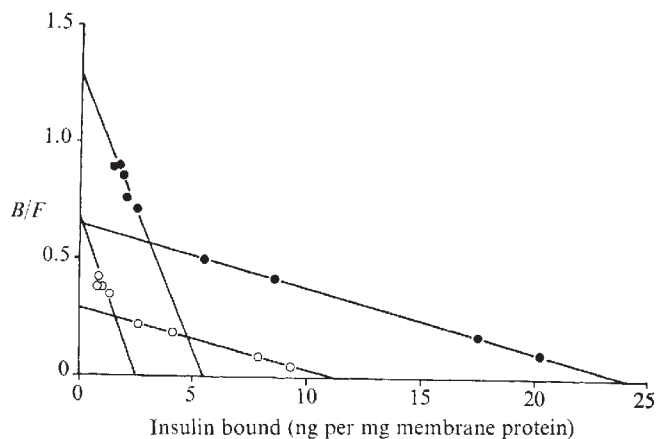


Fig. 1 Scatchard plot of insulin-membrane interaction in two representative preparations from 10 control (○) and 10 diabetic (●) animals. Incubation was performed at 25 °C for 20 min with 0.2 mg of membrane protein per ml and insulin concentrations between 4 and 400 ng ml⁻¹. Insulin degradation was 1.3% in these conditions. Nonspecific binding, that is labelled hormone not displaced by 1 U ml⁻¹ has been subtracted.

heterogeneity of the labelled hormone¹¹ are possible, the essential observation—of an abnormally high binding capacity in membranes from diabetic hamster—remains the same.

Severely diabetic Chinese hamsters have abnormally low plasma and pancreatic insulin, a low insulin response to a glucose load and, consequently, low glucose tolerance⁴. Together with a tendency for ketosis⁴, these features resemble juvenile diabetes in man, and the diabetic Chinese hamster provides an interesting model for this type of the disease. Apparently the liver adapts to the decreased hormone concentration by raising the number of receptors in the plasma membrane, whereas, conversely, in the hyperinsulinaemic C57BL ob/ob mouse¹ as well as in the KK-mouse¹², the total receptor capacity is lower than normal. Our data therefore support the hypothesis that the hormone level controls the number of receptor sites in the liver cell.

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- Kahn, C. R., Neville, D. M., and Roth, J., *J. biol. Chem.*, **248**, 244–250 (1973).
- Archer, J. A., Gordon, P., Gavin, J. R., Lesniak, M. A., and Roth, J., *J. clin. Endocr. Metab.*, **36**, 627–633 (1973).
- Gavin, J., Roth, J., Neville, D. M., De Meyts, P., and Buell, D. N., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 84–88 (1974).
- Gerritsen, G. C., and Dulin, W. E., *Diabetologia*, **3**, 74–84 (1967).
- Neville, D. M., *Biochim. biophys. Acta*, **154**, 540–552 (1968).
- Solyom, A., and Trams, E. G., *Enzyme*, **13**, 329–372 (1972).
- Maeno, H., and Greengard, P., *J. biol. Chem.*, **247**, 3269–3277 (1972).
- Kahn, C. R., Freychet, P., Roth, J., and Neville, D. M., *J. biol. Chem.*, **249**, 2249–2257 (1974).
- De Meyts, P., Roth, J., Neville, D. M., Gavin, J. R., and Lesniak, M. A., *Biochim. biophys. Res. Commun.*, **55**, 154–161 (1973).
- House, P. D. R., *FEBS Lett.*, **16**, 339–342 (1971).
- Taylor, S. I., *Biochemistry*, **14**, 2357–2361 (1975).
- Kern, P., Picard, J., Caron, M., and Veissiere, D., *Biochim. biophys. Acta*, **389**, 281–289 (1975).

Synaptic coupling into the production and storage of a neuronal memory trace

AN adequate model for the nature of the memory trace at the neuronal level should provide for a sequence of physiological mechanisms which would include 'read-in' of the neuronal change, 'storage' processes that impart an enduring quality, and 'read-out' of the change in the form of an altered neuronal response to an appropriate neural input. The framework for such a neuronal model seems to be provided by the features of a novel heterosynaptic interaction in a mammalian sympathetic ganglion. It had been found previously that one synaptic transmitter (the catecholamine dopamine, DA) could produce a specific and enduring enhancement, lasting for at least several hours, of the subsequent responses to another synaptic transmitter (acetylcholine, ACh)¹. We have now found that the retention of this DA-induced change can be disrupted by cyclic GMP in a strikingly time-dependent manner that is similar, in principle, to the well known disruptability feature of the memory storage process^{2,3}. Additional evidence indicates that the production and storage of the enduring change initiated by DA are mediated intraneuronally by cyclic AMP. We present here a scheme which outlines the cellular pathways postulated to carry out the sequence of events from 'read-in' through to 'read-out'.

The rabbit superior cervical ganglion was studied *in vitro* at ~22 °C, in a superfusion chamber with a high resistance sucrose-gap along a section of postganglionic nerve between the recording leads¹. The section containing the ganglion proper had a volume of 0.3 ml and was perfused continuously with Krebs-Ringer solution at about 1 ml min⁻¹. The test response consisted of the slow depolarisation of the ganglion cells produced by the muscarinic agonist acetyl- β -methylcholine (MCh)⁴. The variable complicating factor of a hyperpolarising component in the response to such cholinergic agonists, which is due to the action of DA released from an interneurone, was eliminated by a pre-treatment with the muscarinic agent bethanechol; the latter depletes the ganglion of its functionally-releasable DA⁵. To observe the DA-modulatory action, the slow depolarising response to a test dose of MCh is obtained before and at various times after a brief superfusion with a single supra-maximal dose of DA (for example, Fig. 1, compare tracings *a*, *b* with *c*, *d*). Enhancement of the cholinergic responses can persist unabated for the duration of the ganglion's survival *in vitro*, that is more than for 3 h.

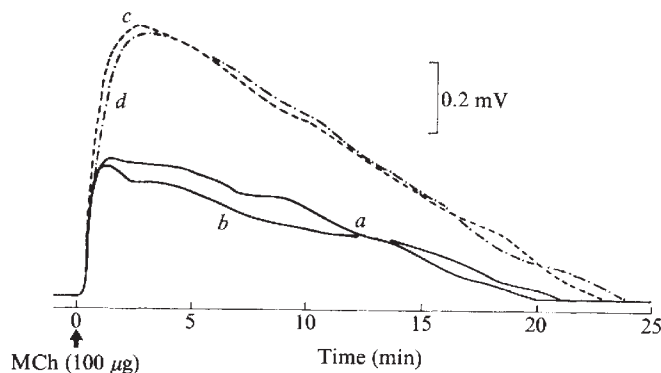


Fig. 1 Modulatory enhancement of methacholine (MCh)-depolarisation after dopamine (DA), and the absence of any effect on this when cyclic GMP was applied 30 min after DA. Each tracing is the slow depolarising response to a single injection of MCh (100 µg). *a* and *b*, Controls before DA. *c*, MCh response at 60 min after a single injection of DA (50 µg), with an interposed addition of cyclic GMP (50 µM for 8 min) which began at 30 min after DA. *d*, MCh response at 60 min after a second dose of DA, with no interposed treatment by cyclic GMP.

The usual DA-produced enhancement of the MCh-depolarisations could be prevented if dibutyryl cyclic GMP (50 µM) was temporarily added (for 8 min) to the superfusing fluid. Disruption of the expected modulatory effect could be complete even when the onset of cyclic GMP treatment was delayed for up to 4 min after the DA injection (Fig. 2, compare tracings *a*, *b* with *c*). A succeeding, second exposure to DA, which was not followed by treatment with cyclic GMP, could still produce the usual later enhancement of MCh-depolarisations (Fig. 2, compare tracing *c* with *d*, *e*). Guanosine 5'-monophosphate (non-cyclic GMP) could produce some small disruptive effects; guanosine, dibutyryl cyclic AMP and adenosine 5'-monophosphate (non-cyclic AMP) had no disruptive action. Additionally, an 8-min treatment with Ringer containing 10 mM K⁺ simulated the depolarisation that is often elicited by the dibutyryl cyclic GMP itself, but it had no disruptive effect on the DA-modulatory action.

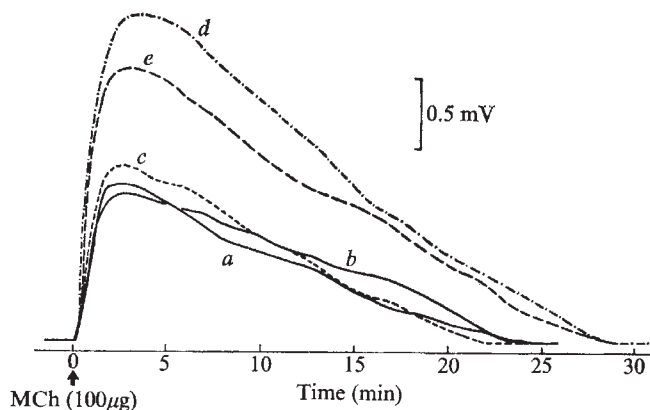
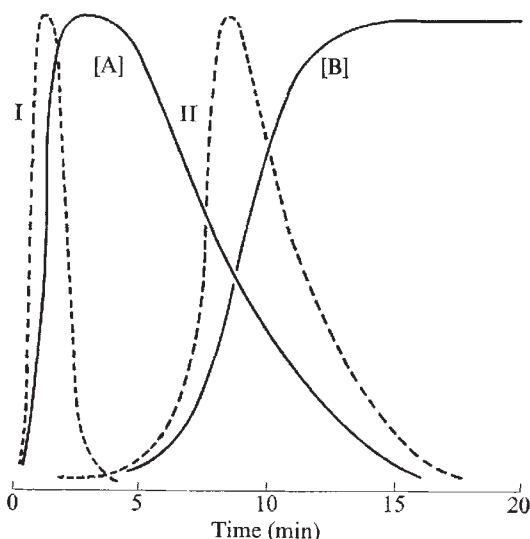


Fig. 2 Disruption of DA-modulatory effect on MCh responses when treatment with cyclic GMP was begun at 4 min after DA. *a* and *b*, Control MCh responses. *c*, MCh response at 35 min after the single DA dose (50 µg), with an interposed addition of cyclic GMP (50 µM for 8 min) which began at 4 min after DA. *d*, MCh response 35 min after a second dose of DA, with no interposed treatment with cyclic GMP. *e*, Additional MCh response at 50 min after that in *d*, with no interposed treatments at all. (The bulk of the enhancement effect is still evident in *e*; but the smaller response in *e* relative to *d* is an example of some late ganglionic deterioration, not of a fall-off in the effect.)

When treatment with cyclic GMP was delayed for periods longer than 4 min after DA, progressively lesser disruptions occurred. With delays of greater than 10–15 min, there was no disruption; that is, retention of the modulatory effect of DA was then unaffected by cyclic GMP (Fig. 1). An initial manifestation of the modulatory enhancing action, which could be observed when DA was injected during a continuous superfusion of MCh (0.5 µM), was also unaffected by the presence of cyclic GMP.

These features of the actions by cyclic GMP suggest that, after the initial modulating change is induced by DA, a 'storage' process develops which leads to a more durable form of modulatory change (see Fig. 3); in this view, the storage process (but not the initial or the durable products) can be antagonised or disrupted by cyclic GMP. On the basis of further evidence (below) we also propose that cyclic AMP is the intracellular mediator for processes I and II in Fig. 3, that is, for producing the DA-enhancing action and its 'storage'. This function would be additional to the already proposed role^{6–8} for cyclic AMP as a mediator of a direct inhibitory action of DA, which had also been previously described^{1,4}.

The ability of cyclic AMP to produce an enduring enhancement of the depolarising response to MCh was demonstrated by substituting a treatment with dibutyryl cyclic AMP (1 mM for 6–10 min) for the dose of DA, in



experiments similar to those in Figs 1 and 2. The enhancement produced by cyclic AMP could occlude fully the production of any further enhancement by a subsequent dose of DA; and it could also be disrupted by cyclic GMP in a time-dependent manner. It may be argued that the similarity of actions by cyclic AMP and DA is simply due to DA in both cases, with cyclic AMP producing a pre-synaptic release of DA (a possibility raised by reports for certain other cases⁹). This possibility seems, however, to be excluded here. (1) As noted, the ganglia were almost completely depleted of their functionally-releasable DA by a

Fig. 3 Time curves for processes and cell products proposed as components in the DA-modulatory action. Process I would involve reactions that lead to the molecular changes which are responsible for the initial enhancement of the slow depolarising response to ACh (the latter is equivalent to the slow excitatory postsynaptic potential, or s-e.p.s.p.). The nature and site of these DA-induced molecular changes, in the unique sequence of events that begins at the ACh-muscarinic receptor and ends with the s-e.p.s.p. response⁴, are not yet known; but we may refer to the relevant altered molecular state [A] as the 'product' of process I. Without further processing, [A] would gradually decay over a period of some minutes (certainly less than 30 min), and the responsiveness of the neurone to ACh (or MCh) would revert to the resting control level that existed before the DA modulating action. Cyclic GMP seems to have no effect on process I, or on product [A] and its effectiveness. Process II represents a storage-type of reaction which converts [A], or a separate precursor of [A], into the more durable altered state [B].

suitable pretreatment^{4,5}. (2) Treatment of a ganglion for 50 min with monobutyl cyclic AMP (in the presence of theophylline and α -methyl-*p*-tyrosine) produced no evidence of depletion of functional DA, in contrast to that seen with a briefer bethanechol treatment. The additional important requirement, for a proposal that cyclic AMP mediates the DA-modulatory action, is that DA (or a suitable input of preganglionic nerve impulses) be capable of eliciting an adequate increase in cyclic AMP in the ganglion cells. This requirement has already been met adequately by previous findings^{7,8,10}.

The scheme presented in Fig. 4 provides a theoretical outline of the full sequence of the physiological actions involved in producing, storing, and 'reading-out' the enduring modulatory effects on the s-e.p.s.p. responses to cholinergic inputs. We do not exclude the possibility that

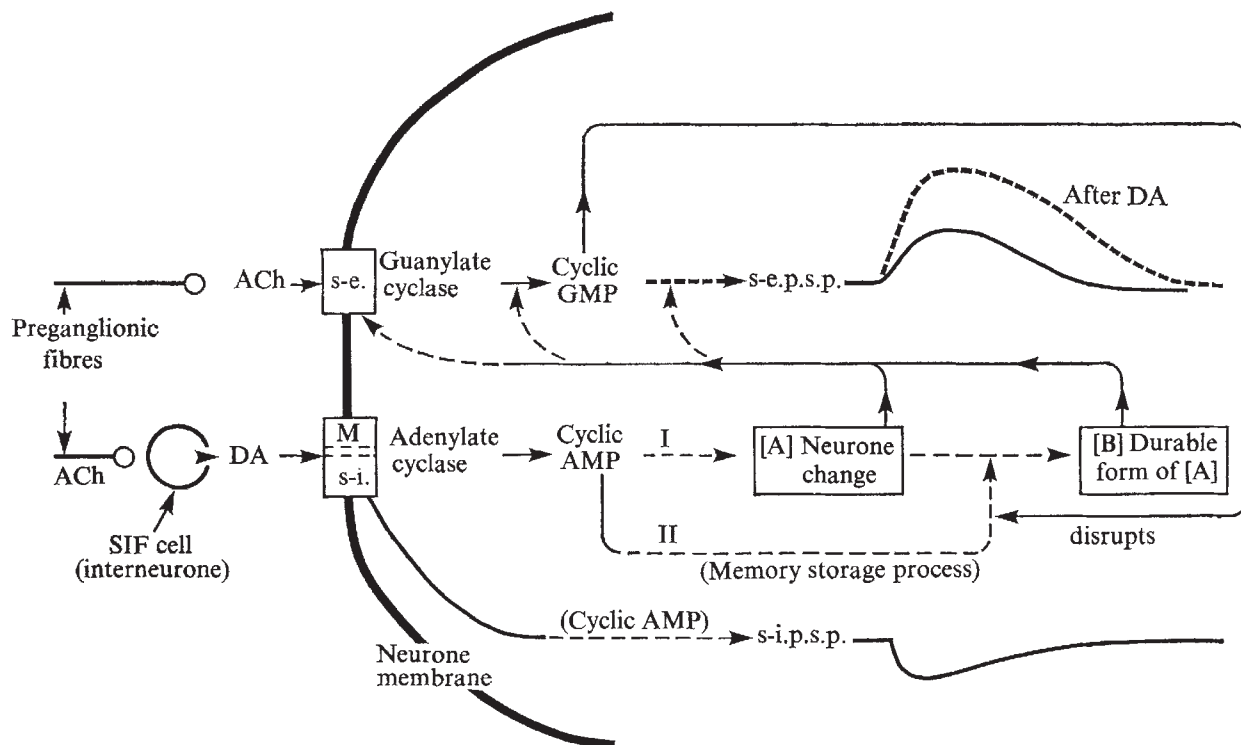


Fig. 4 Schema of pathways for synaptic inputs and intracellular mediators involved in the responses to DA and ACh, for a principal neurone (or ganglion cell) in the rabbit superior cervical ganglion. The s-e.p.s.p. is a slow depolarising change in the membrane potential in response to ACh acting at a muscarinic type of membrane receptor (s-e.); it is represented both before and after (dashed line) its enhancement by a modulatory action of DA. The s-i.p.s.p. is a slow hyperpolarising change in membrane potential produced by a direct action of DA. DA is released by a special type of interneurone⁹ (the small intensely-fluorescent, SIF, cell as seen with the Falck-Hillarp technique), in response to preganglionic impulses or to an applied muscarinic

agonist of ACh⁴. The two postsynaptic actions of DA¹ are designated as M for the modulatory one and s-i. for the hyperpolarising response or s-i.p.s.p.; the actions could be mediated by a single or by two separate membrane receptors. Persisting enhancement of the s-e.p.s.p. response after exposure to DA would be somehow dependent on neuronal change [A] and its more durable form [B]. The question of the precise sites (in the sequence of reactions leading to the s-e.p.s.p. response) at which the modulating neuronal change is operative, is at present being investigated and is represented by a set of dashed arrows. (The proposals for a guanylate cyclase-cyclic GMP role in mediation of s-e.p.s.p. response, and for cyclic AMP as mediator of s-i.p.s.p., were made previously^{5-8,12}.)

cyclic AMP may mediate the direct inhibitory action of DA⁶⁻⁸, in addition to the enduring modulatory action by DA. On the other hand, the present evidence that cyclic AMP can mediate the DA-modulatory action suggests that this role could be an important one for the DA- (and possibly the noradrenaline-) coupled increases of cyclic AMP in various brain neurones¹¹.

The DA-modulating action in the sympathetic ganglion provides a model in which the development of a shorter term memory, a memory storage process, and the production and retention of a longer term memory trace, are all accomplished within the same single neurone and are all initially set into motion by the same intracellular messenger (cyclic AMP) following a single brief type of synaptic input. Repetitive synaptic actions fed in by reverberating circuits of nerve cells have been postulated to underlie short term memory and memory storage^{2,3}, but these are neither necessary nor available in the ganglion. In the present model, a heterosynaptic interaction takes place between two types of synaptic inputs to the same neurone; the memory trace is initiated by a brief (dopaminergic) input in one synaptic line, while the 'read-out' of the memory consists simply in the enhanced ability of the postsynaptic unit to produce its specific response to another (cholinergic) synaptic input. This arrangement provides for a 'learned' change in the response to one input as a result of an 'experience' previously carried by way of another input.

This proposed model does not in itself provide for the specificity of content of a given memory. Some prevalent hypotheses have attempted to provide for such specificity by postulating a specific memory trace¹³, but they have suffered from an inadequacy in providing for both the 'read-in' and 'read-out' of the putatively encoded information. Perhaps the specific nature of a given engram could be sought outside the confines of the individual cell in group patternings, but based on the acquisition, by some individual neurones in these groups, of a memory trace that is itself not so specific. The individual neuronal memory portion of such an engram would then acquire a more general character common to many neurones. The view that our proposed model or variants of it may in fact have some general significance receives some support from indirect lines of evidence; the latter have added monoaminergic¹⁴ to the cholinergic pathways already known to widely innervate the forebrain, and have indicated that cholinergic¹³ and various monoaminergic transmitters¹⁵ may have roles in long lasting functions of the central nervous system, including those in learning and memory.

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- ¹ Libet, B., and Tosaka, T., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 667-673 (1970).
- ² Gerard, R. W., *Am. J. Psychiat.*, **112**, 81-90 (1955).
- ³ McLaugh, J. L., *Science*, **153**, 1351-1358 (1966).
- ⁴ Libet, B., *Fedn Proc.*, **29**, 1945-1956 (1970).
- ⁵ Libet, B., and Owman, Ch., *J. Physiol., Lond.*, **237**, 635-662 (1974).
- ⁶ McAfee, D. A., and Greengard, P., *Science*, **178**, 310-312 (1972).
- ⁷ Keabian, J. W., and Greengard, P., *Science*, **174**, 1346-1348 (1971).
- ⁸ Kalix, P., McAfee, D. A., Schorderet, M., and Greengard, P., *J. Pharmac. exp. Ther.*, **188**, 676-687 (1974).
- ⁹ Goldberg, A. L., and Singer, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 134-141 (1969).
- ¹⁰ Aleman, V., Bayon, A., and Molina, J., *Adv. behav. Biol.*, **10**, 115-124 (1974).
- ¹¹ Stone, T. W., Taylor, D. A., and Bloom, F., *Science*, **187**, 845-847 (1975).
- ¹² Keabian, J. W., Steiner, A. L., and Greengard, P., *J. Pharmac. exp. Ther.*, **193**, 474-488 (1975).
- ¹³ Deutsch, J. E., Ed., *The Physiological Basis of Memory* (Academic, New York, 1973).
- ¹⁴ Hökfelt, T., Fuxe, K., Johansson, O., and Ljungdahl, A., *Eur. J. Pharmac.*, **25**, 108-112 (1974).
- ¹⁵ Kety, S., *Adv. behav. Biol.*, **4**, 65-80 (1972).

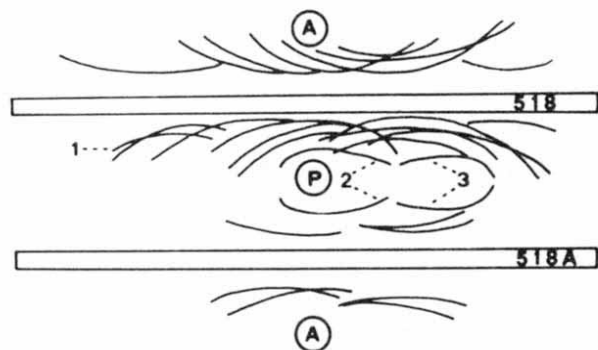
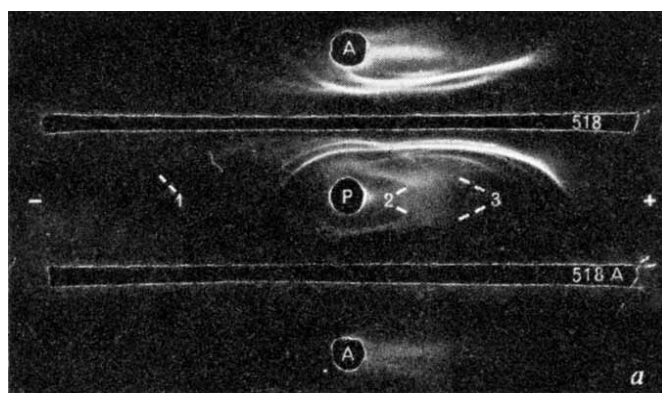
Molecular anisotropy of the early *Drosophila* embryo

It is generally accepted that cellular differentiation depends chiefly on differential gene activity¹. The model of differential gene activity implies the existence of different inducers/repressors activating/repressing different genes. Indirect evidence for such molecules in *Drosophila* comes from experiments of Illmensee and Mahowald² who showed that the information for primordial germ cell determination is in the cytoplasm of the posterior pole of the egg and that when this cytoplasm is transferred to the anterior pole so is the information. Their experiments suggest that genes are regulated not only in time but also in space, possibly by molecules distributed unequally in the developing system³. We have analysed *Drosophila* eggs for such molecules.

Kock and Heinig⁴ and Nuneman and Moser⁵ first approached the problem of unequally distributed molecules in insect eggs by microelectrophoresis and immunoelectrophoresis of parts of the egg of *Achaeta domestica*. They found quantitative differences between the anterior and posterior halves of the egg which probably depend on the unequal distribution of yolk platelets. We have approached this problem in *Drosophila melanogaster* by first developing a technique for producing extracts of different parts of the egg and then producing antisera against these extracts.

Eggs were collected on agar plates seeded with fresh bakers' yeast from population cages containing about 10,000 flies. Eggs (500-700) were oriented vertically on a flat surface with the posterior poles lying in the same plane. When the eggs had reached the desired developmental stage they were frozen in a glass frame. The dimensions of

Fig. 1 *a*, Immunoelectrophoresis of 20% posterior (P) and 80% anterior (A) pole extracts. Protein concentration 40 mg ml⁻¹. Bands 1, 2 and 3 were found only in P. Antiserum 518 and antiserum 518A which was absorbed with A. Note that bands 2 and 3 remain unchanged with the absorbed antiserum while other bands either disappear or become weaker. *b*, Tracing of immunoelectrophoresis plate.



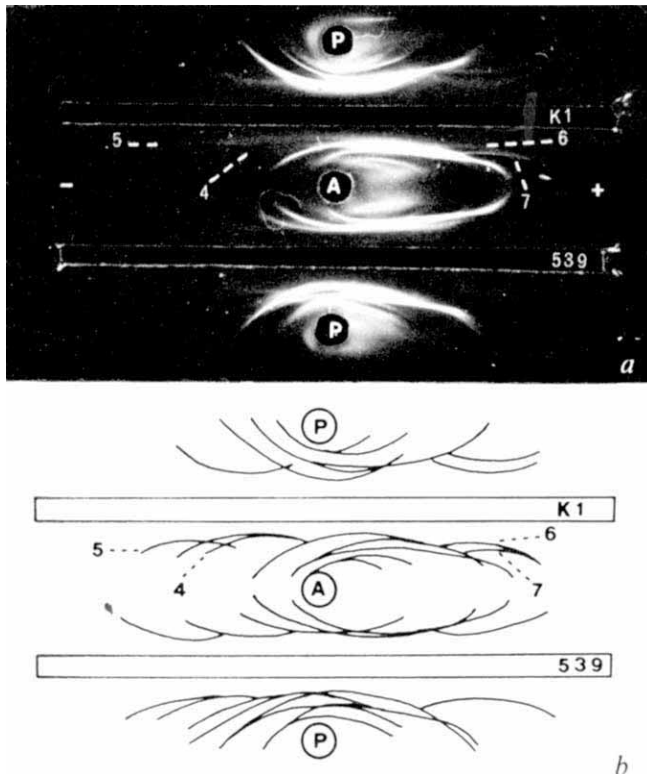


Fig. 2 *a*, Immunoelectrophoresis of 30% anterior (A) and 70% posterior (P) pole extracts. Protein concentration 40 mg ml^{-1} . Band 5 was detected only in A. Bands 4, 6 and 7 show a significant quantitative variation between A and P where they were more concentrated. Antiserum K1 and antiserum 539 also anti-0-4 h embryo. *b*, Tracing of immunoelectrophoresis plate.

the frames were such as to allow different proportions of the eggs to protrude above the surface. The protruding portions of the eggs were cut off in a cryostat microtome and the two parts of the eggs collected in different tubes. By adjusting the dimensions of the frame, the posterior 20%, 30%, 40% and so on, as estimated by the relative protein concentration⁶, could be cut off.

The preparation of antisera and other immunological techniques were as reported previously⁷. The extracts were prepared by sonicating the cut embryos. Nine different antisera were used and only three of these showed differences in the antigen patterns of the extracts from different parts of the egg.

Antiserum 518 (0-4-h embryo extract, 30-40% ammonium sulphate fraction) showed antigens present in the posterior pole but not in the anterior pole represented by precipitin arcs 1, 2 and 3 (Fig. 1). These antigens were detected in the posterior 20% of the egg. If larger portions of the posterior part were taken, however, the weaker antigens could not be detected and the stronger ones became weaker, as would be expected if the antigens were located in the extreme posterior cytoplasm and were diluted with anterior cytoplasm lacking them. When the antiserum was absorbed with an extract of the anterior 80% of the egg and used to analyse extracts of the posterior 20%, arcs 2 and 3, identified as being in the posterior part of the egg, were unchanged on immunoelectrophoresis plates while the other arcs disappeared or were reduced because of the removal of the antibodies by absorption (Fig. 1).

A second antigen showing unequal distribution in the egg was detected with antiserum K1 (0-4-h embryos). Extracts of the anterior 30% and 70% of the egg were compared with extracts of the posterior 70% and 30% respectively. Antigen 5 (Fig. 2) was limited to the anterior 30% of the egg. This antiserum also shows significant quantitative differences.

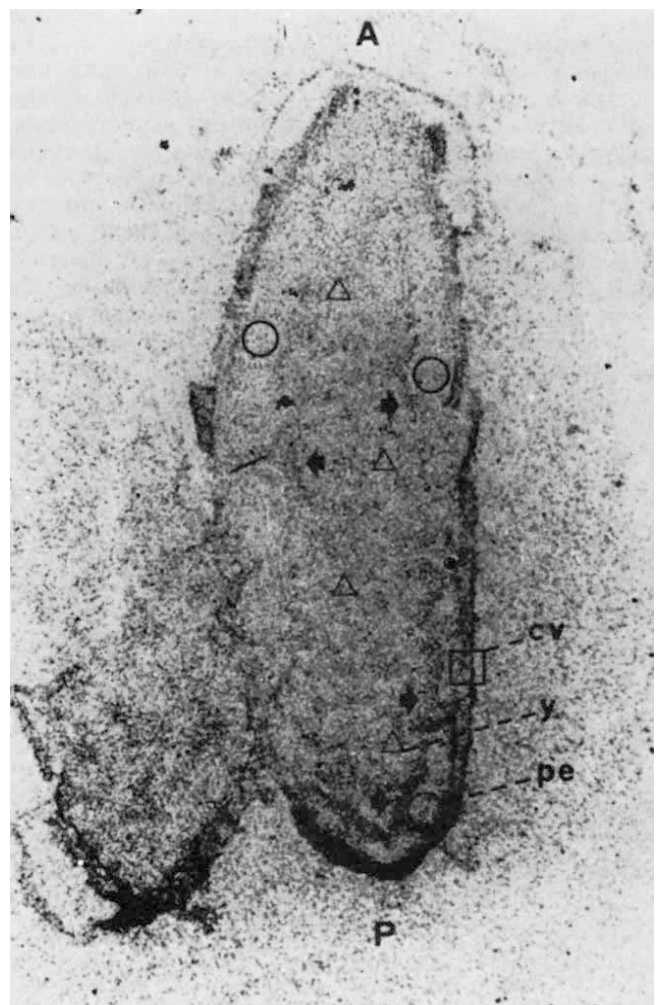
Antiserum 547 was prepared against an extract of the

posterior 30% of the egg and was used to compare extracts of the anterior 30% and 70% of the egg with extracts of the posterior 70% and 30% respectively. One antigen was found in the posterior 30% fraction and not in the anterior 30% or 70% fractions. A second antigen was always associated with the larger portion of the egg irrespective of whether it was the anterior or posterior 70%. We suggest that this antigen is found in the central cytoplasm (including the yolk) and is detected in those extracts with the highest percentage of the cytoplasm, that is, the extracts of the larger part of the egg.

In order to test our proposals that antiserum 547 detected antigens associated with the posterior egg cytoplasm and with the central cytoplasm, as well as more general antigens, we sectioned frozen eggs with a cryostat microtome. The sections were flooded and incubated with antiserum 547, washed with saline and treated with ¹²⁵I-labelled horse anti-rabbit immunoglobulin. Autoradiographs of the washed slides show most of the radioactivity in the posterior pole of the egg and peripherally in the central cytoplasm at the boundary between the periplasm and the yolk mass thus confirming our prediction of the distribution of antigens detected by this antiserum (Fig. 3).

Although no technique allows us to say that a molecule is not in a certain part of the egg, these results demonstrate

Fig. 3 Autoradiograph of a section of 120-min-old egg treated with antiserum 547 followed, after washing, by treatment with ¹²⁵I-labelled horse anti-rabbit IgG. Treatment of similar sections with non-immune serum 547 and horse antibody or with horse antibody alone showed only nonspecific association of radioactivity with the chorion and a low overall background. cv, Chorion and vitelline membrane; pe, periplasm; y, yolky cytoplasm; A, anterior pole; P, posterior pole; arrows, boundary line between the periplasm and the yolky cytoplasm.



that some antigens at least are not distributed homogeneously in the egg cytoplasm. This result is all the more interesting as the antigens studied on double diffusion plates must be soluble antigens which *a priori* might be expected to be distributed homogeneously through the cytoplasm. Insoluble or membrane bound antigens, the heterogeneous distribution of which might be more readily understood, could only have been detected by the autoradiographic study.

Whether this unequal distribution of antigens in the *Drosophila* egg reflects the mosaic nature of the egg or is a trivial consequence of a more important gradient cannot be decided from these studies. The analysis of mutants showing disturbed embryonic development, for example temperature sensitive female sterile mutants, may help to resolve this problem.

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¹ Davidson, E. H., *Gene activity in early development* (Academic, New York and London, 1968).

² Illemensee, K., and Mahowald, A. P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1016–1020 (1974).

³ Gurdon, J. B., *The control of gene expression in animal development* (Clarendon, Oxford, 1974).

⁴ Koch, P., and Heinig, S., *Roux' Arch. Ent. Mech.*, **161**, 241–248 (1968).

⁵ Nunemann, H., and Moser, J. G., *Zool. Anz. Suppl.*, **33**, 113–120 (1970).

⁶ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).

⁷ Roberts, D. B., *Nature*, **233**, 394–397 (1971).

Induction of puffs in *Drosophila* salivary gland cells by mitochondrial factor(s)

It is well established that specific puffs can be induced in the polytene chromosomes of *Drosophila* larvae by treatments such as heat shock^{1–3} and by substances which interfere with

respiration⁴; however, the intracellular signals produced in these conditions for the induction of specific puffs are not known. It has been suggested⁵ that these signal(s) might come from mitochondria. I have investigated this possibility, using *Drosophila hydei*.

After heat shock a mitochondrial suspension was centrifuged and the supernatant injected into the cytoplasm of salivary gland cells. The responses of chromosomal regions 32A, 36A, 48BC and 81B, which are known to be sensitive to heat shock *in vivo* and *in vitro*², were analysed. Table 1 shows the results of analysis of regions 36A and 48BC. Injection of the 'injection medium', followed by 35 min incubation in Poels' medium⁶ caused no significant changes in the relative diameters of these two regions. Microinjection into the salivary gland cells of the supernatant from mitochondria which had been subjected to 37 °C caused a significant increase in the relative diameter of regions 36A and 48BC. The extent of increase at region 36A was 11 %, comparable with that obtained from tissue which had been subjected to heat shock *in vitro*. The increase at region 48BC was 23 % over the normal relative diameter of 1.27, a value greater than that (13 %) from heat-shocked salivary glands. Even larger puffs can be induced at this chromosome region by vitamin B₆ *in vivo*⁷. Injection of the supernatant from mitochondria which were kept at 20 °C failed to induce puffs at these regions. The puff-inducing ability of the mitochondrial supernatant was greatly reduced after boiling for 10 min. This suggests that the puff-inducing agent(s) require a certain structural configuration to be effective, or that they are complexes which can be dissociated by boiling. These puff-inducing agent(s) seem to have relatively high molecular weight as they could not be dialysed out. The possibility that these mitochondrial agent(s) were nonspecific adhering substances of cytoplasmic origin, being modified and released under heat shock, was also tested. Cytoplasm prepared by lightly homogenising tissue in the 'injection medium' followed by centrifuging at 12,000g to remove cell debris and mitochondria, was heated at 37 °C as described for mitochondria, and injected into intact gland cells. At the two concentrations used (about 4 pg and 20 pg protein per cell), no changes were observed in the relative diameters of regions 36A and 48BC.

Figure 1 shows the dose response of the two chromosome regions to the amount of mitochondrial agent(s) injected. The maximum relative diameter of the puff induced at region 36A was attained after injection of about 1.25 pg protein; and at

Table 1 Relative diameter of chromosome regions 36A and 48BC

Experiment	No. of mitochondrial preparations tested	Chromosome region	Relative diameter	t Test
(1) Salivary gland cells injected with injection medium incubated in Poels' medium for 35 min at 20 °C	—	36A 48BC	1.00 ± 0.002*(46) 1.27 ± 0.03 (36)	
(2) Salivary gland in Poels' medium for 35 min, at 37 °C	—	36A 48BC	1.12 ± 0.01 (73) 1.44 ± 0.03 (74)	
(3) Salivary gland cell injected with supernatant from mitochondria kept at 20 °C for 35 min	8	36A 48BC	1.01 ± 0.01 (62) 1.31 ± 0.03 (61)	Compared with (1), <i>P</i> > 0.03 <i>P</i> > 0.02
(4) Salivary gland cell injected with supernatant from mitochondria kept at 37 °C for 35 min	10	36A 48BC	1.11 ± 0.01 (84) 1.56 ± 0.02 (68)	Compared with (1), <i>P</i> < 0.005 <i>P</i> < 0.005
(5) As in (4), but supernatant was heated at 100 °C for 10 min	3	36A 48BC	1.02 ± 0.01 (26) 1.35 ± 0.03 (27)	Compared with (4), <i>P</i> < 0.005 <i>P</i> < 0.005
(6) As in (4), but supernatant was dialysed overnight	4	36A 48BC	1.10 ± 0.03 (53) 1.53 ± 0.03 (49)	Compared with (4), <i>P</i> > 0.2 <i>P</i> > 0.2

Third-instar larvae of *D. hydei* reared under standardised conditions⁸ were used. Mitochondria were prepared⁹ from 300–400 mg mass isolated¹⁰ salivary glands. The well washed (by repeated suspension and centrifugation) mitochondria, suspended in 0.8 ml of 'injection medium' (0.154 M KCl; 1.0 mM EDTA, pH 7.1), were divided into two equal portions. To simulate heat shock, one portion was kept at 37 °C for 35 min, the other portion at 20 °C. After heat shock the mitochondrial suspensions were centrifuged at 27,000g for 100 min at 4 °C. The supernatant is referred to as mitochondrial supernatant. Microinjection was carried out as before¹¹. The salivary glands, isolated in Poels' medium⁶, were submerged in the same solution for microinjection. Between eight and 15 cells at the distal end of each gland were injected each with 3–5 × 10⁻⁵ µl of mitochondrial supernatant, the sister gland was used as control. For the purposes of comparison, the amount of material injected was expressed on a protein basis (2–4 pg per cell). Protein concentration was determined by absorption at 230 nm, thus the absolute amount of protein injected per cell is likely to be overestimated. After injection the glands were incubated in Poels' medium⁶ at 20 °C for 35 min, then fixed and stained with aceto-orcin; 6–8 injected cells were cut off and squashed. The puffing activity of the chromosome regions was expressed as diameter relative to a reference chromosome band which remained inactive under various conditions. *Mean ± s.e.m. Figures in parentheses indicate number of cells measured.

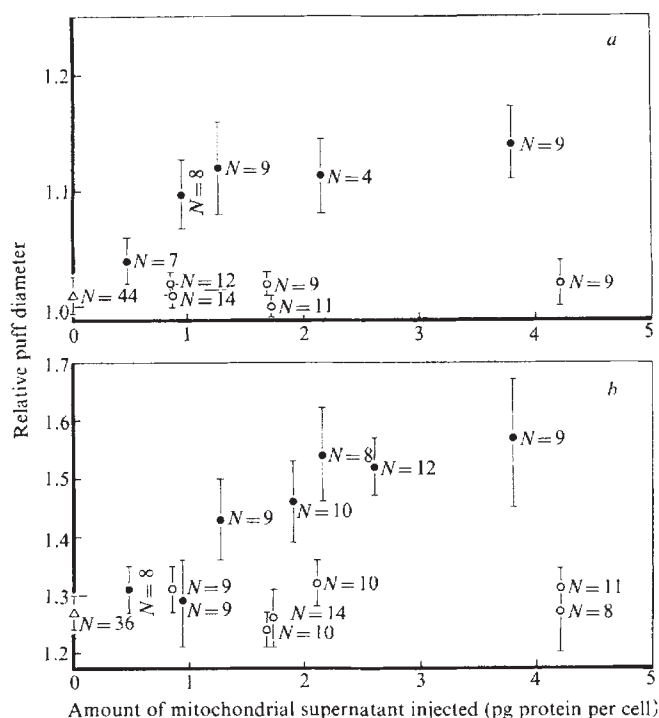


Fig. 1 Response of chromosome regions 36A (a) and 48BC (b) to injection of various amounts of mitochondrial supernatant from heat treated (●) and control (○) mitochondria. △, Cells injected with injection medium. Vertical bars represent s.e.m. N, Number of cells measured.

region 48BC at 2 pg protein per cell. With larger amounts no further substantial increases were observed.

Chromosome region 32A failed to respond to microinjection of the supernatant from the treated mitochondria. Region 81B showed only slight activity and so accurate comparison was not possible. The absence of puff formation at region 32A suggests that the simulation of heat shock of mitochondria *in vitro* does not have the same effect on the mitochondria as it would have in intact tissue, and that more than one puff-inducing agent is required for the induction of all four temperature puffs. The chemical nature of the mitochondrial agent(s) has not been established.

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- Ritossa, F., *Experientia*, **18**, 571-572 (1962).
- Berendes, H. D., Van Breugel, F. M. A., and Holt, T. K. H., *Chromosoma*, **16**, 35-46 (1965).
- Ashburner, M., *Chromosoma*, **31**, 356-376 (1970b).
- Leenders, H. J., and Berendes, H. D., *Chromosoma*, **37**, 433-444 (1972).
- Leenders, H. J., Berendes, H. D., Helmsing, P. J., Derksen, J., and Koninkx, J. F. G. J., *Sub-cell. Biochem.*, **3**, 119-147 (1974).
- Poels, C. L. M., *Cell Differentiation*, **1**, 63-78 (1972).
- Leenders, H. J., Derksen, J., Maas, P. M. J. M., and Berendes, H. D., *Chromosoma*, **41**, 447-460 (1973).
- Mitchell, H. K., and Mitchell, A., *Drosophila Inf. Serv.*, **39**, 135-137 (1964).
- Koninkx, J. F. G. J., Leenders, H. J., and Birt, L. M., *Exp Cell Res.*, **92**, 275-282 (1975).
- Boyd, J. B., Berendes, H. D., and Boyd, H., *J. Cell Biol.*, **38**, 369-376 (1968).
- Brady, T., Berendes, H. D., and Kuipers, A. M. C., *Molec. Cell Endocr.*, **1**, 249-257 (1974).

Rapid loss of a novel phenylalanyl-tRNA on germination of *Neurospora crassa* conidia

SEVERAL theories attempt to explain the control of protein synthesis, and thus development at the level of transfer RNA^{1,10}. We present evidence for changes in the isoaccepting species of

phenylalanyl-tRNA during germination and growth of the fungus *Neurospora crassa*.

The tRNAs and aminoacyl-tRNA synthetases were isolated from conidia and mycelia as described in the legend of Fig. 1. The conidial tRNA preparation was esterified with ³H-phenylalanine using the conidial synthetase, whereas ¹⁴C-phenylalanine was used in the analogous mycelial system. The pattern shown in Fig. 1 was obtained on elution of the tRNAs from a benzoylated DEAE cellulose (BD-cellulose) column. The data were treated according to the method of Shearn and Horowitz⁸, thus providing a direct comparison of the tRNA species even though they are labelled with different forms of radioactivity. Mycelial and conidial cultures were shown to contain two and three phenylalanyl-tRNAs, respectively. In addition to monitoring the radioactive amino acids, fluorescence and absorbancy values were also determined. Although the latter two measurements were performed in subsequent experiments, they are presented only in Fig. 1. Fluorescence measurements enable one to distinguish between the cytoplasmic and mitochondrial species of phenylalanyl-tRNA. The cytoplasmic species contain a fluorescent base while mitochondrial species do not (W. E. Barnett, personal communication). Those phenylalanyl-tRNAs which elute late in the salt gradient (fractions 90-100) do not fluoresce and are presumably of mitochondrial origin. The cytoplasm of conidia contains two species of phenylalanyl-tRNA (Phe-tRNA_I^c and phe-tRNA_{II}^c), whereas the cytoplasm of mycelia contains only one (Phe-tRNA_I^m).

When the conidia are incubated in a medium which supports germination and growth, the minor species of phenylalanyl-tRNA (Phe-tRNA_{II}^c) disappears within 3 h. Although tRNAs are normally stable, their rapid loss is not unknown⁹.

The levels of methyl-group acceptance are reported¹¹ to vary in conidial and mycelial populations of tRNA. To test the possibility that the loss of Phe-tRNA_{II}^c may result from its conversion by methylation to Phe-tRNA_I^c, the following experiments were performed. Preparations of either conidial or mycelial tRNAs were first esterified with ³H-phenylalanine, then subjected, *in vitro*, to methylation using ¹⁴C-S-adenosyl-methionine as the methyl donor in the presence of the nuclease competitive inhibitor 2',3'-cytidine monophosphate (2',3'-CMP). Control experiments involved incubation of the esterified tRNAs in the methylation reaction mixture minus the methyl donor and 2',3'-CMP.

Figure 2a shows the pattern of methyl-group acceptance in a population of conidial tRNAs. Because the Phe-tRNA_{II}^c does not disappear, conversion by methylation is ruled out as a mechanism. Since the levels of the two Phe-tRNA species resolved by the BD-cellulose chromatography were quantified by amino acid charging saturation experiments as well as by fluorescence measurements, the differences in methyl group acceptance between the two forms indicates that Phe-tRNA_{II}^c is undermethylated with respect to Phe-tRNA_I^c. Methylation of tRNAs other than Phe-tRNA results in an accumulation of these ¹⁴C-labelled compounds in the 0.9 M NaCl wash. When the conidial tRNAs were incubated in the methylase reaction mixture minus the methyl donor and 2',3'-CMP, a measure of endogenous nuclease activity was obtained. As a result of the incubation, Phe-tRNA_I^c remained at its initial level but the Phe-tRNA_{II}^c level was decreased (Fig. 2b). Furthermore, some of the ³H-phenylalanine eluted very early in the salt gradient and the remainder was removed in the 20% ethanol wash. The products of the nuclease attack were found in the 20% ethanol wash, whereas the radioactivity which appeared early in the salt wash was free phenylalanine resulting from deacylation of the charged Phe-tRNAs due to the high pH of the reaction mixture. Figure 2c and d is analogous to the experiments described above except that the mycelial tRNA preparation was used. The important features of these latter two experiments are: (1) only a small amount of ¹⁴C-methyl label is accepted by the mycelial tRNA^{Phe} suggesting this species is fully methylated *in vivo*; (2) the level of mycelial tRNA^{Phe} is not altered by incubation in the methylase reaction mixture minus the methyl donor and

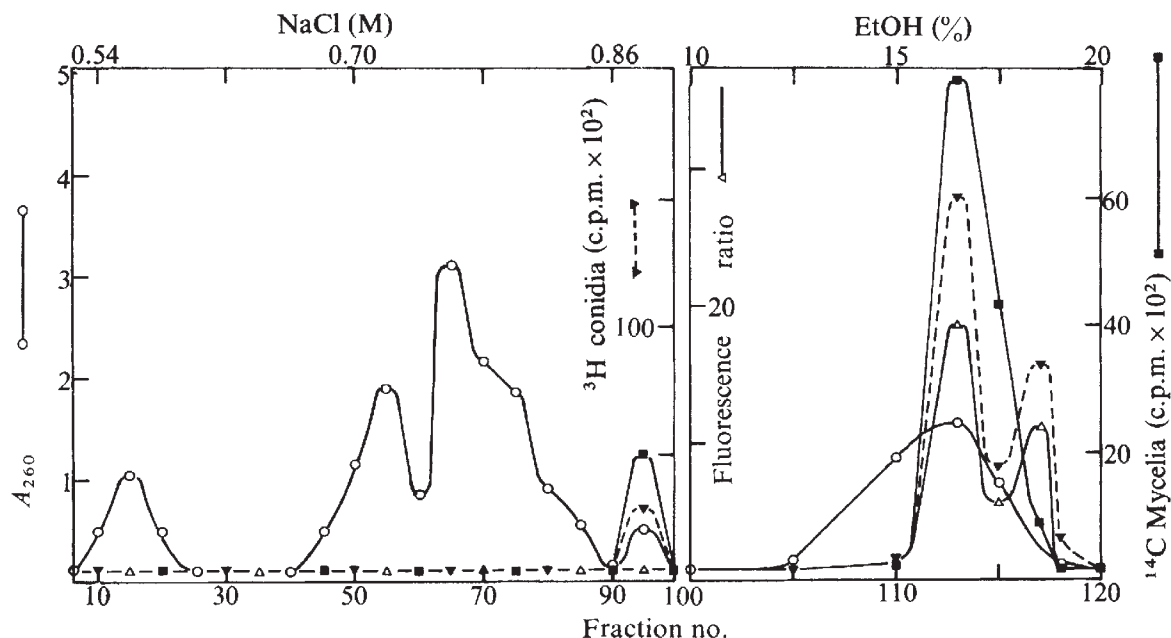


Fig. 1 BD-cellulose column chromatography of phenylalanyl-tRNAs from *N. crassa* conidia and mycelia. Conidia and mycelia, from the wild type strain 74-OR-23-1A, were used as sources of tRNA and aminoacyl-tRNA synthetase. The tRNA was extracted by a modified method of Holley *et al.*³. The reaction mixture contained 20 ml of 0.1 M Tris buffer, pH 7.4, containing 0.001 M MgCl_2 and 0.005 M KCl and 15 ml of water-saturated phenol per 10 g wet weight of cells. The aminoacyl tRNA synthetases were prepared using the method of Haskinson and Khorana⁴. The reaction mixture for the assay of amino acid acceptance was patterned after the methods of Reeves *et al.*⁶ and of Shearn and Horowitz⁷. The final concentrations of reactants in the 2.5-ml assay were: Tris-HCl, pH 7.5, 50 mM; MgCl_2 , 15 mM; NaEDTA, 1 mM; Na ATP, 5 mM; ^{14}C or ^3H amino acid, 30 μM (specific activity was 50 $\mu\text{Ci } \mu\text{mol}^{-1}$ for ^{14}C and 250 $\mu\text{Ci } \mu\text{mol}^{-1}$ for ^3H); 40–60 A_{260} units of tRNA per ml; and synthetase preparation (2.0 mg ml^{-1}). BD-cellulose column chromatography was carried out as described by Gilliam *et al.*². The column was equilibrated with a buffer containing 0.6 M NaCl, 0.01 M MgSO_4 and 0.01 M NaAc, pH 4.5. The tRNAs were eluted with a 400-ml linear gradient of 0.6 M to 0.9 M NaCl in the above buffer. After completion of the salt gradient, a 200-ml, 10–20% ethanol gradient in the 0.9 M NaCl buffer was applied. Fractions of 4 ml were collected and assayed for radioactivity, absorbance and fluorescence. The fluorescence ratio of 1 ml in 0.1 M K_3PO_4 was measured in a Beckman ratio fluorometer, model 772. The excitation wavelength was 310 nm and fluorescence was measured in the 410–460-nm range.

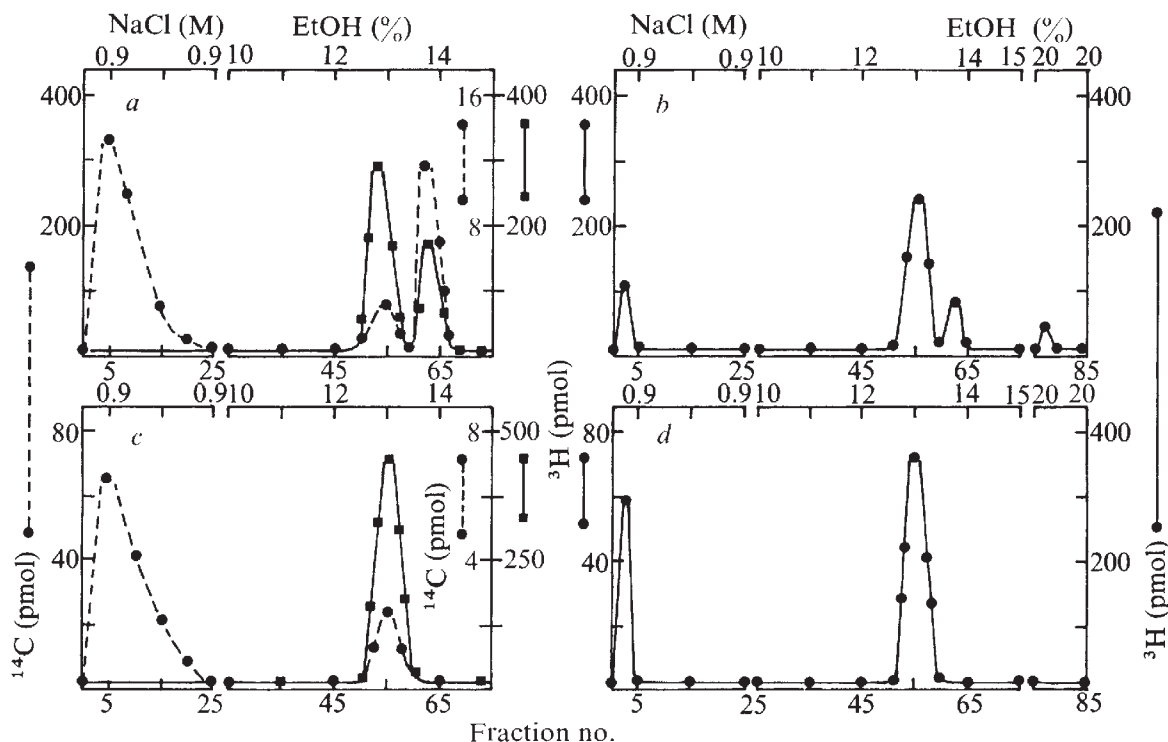


Fig. 2 Effect of methylation on phenylalanyl-tRNA isolated from *N. crassa* conidia and mycelia. The assay for methyl group acceptance by tRNA was patterned after experiments described by Wong *et al.*¹¹. The tRNA was esterified and prepared for BD-cellulose chromatography as described in the legend of Fig. 1; however, before application to the column, the tRNA was added to the following reaction mixture: Tris-HCl, pH 7.0, 20 μM ; mercaptoethanol, 2 μM ; MgCl_2 , 2 μM ; tRNA, 100 μg ; enzyme, 0.5 mg; ^{14}C -methyl S-adenosyl-methionine, 2 μM (specific activity 50 $\text{mCi } \text{mmol}^{-1}$). The tRNA preparation was applied to the BD-cellulose column, washed with 0.9 M NaCl buffer, and then eluted with a 10–15% ethanol gradient in 0.9 M NaCl buffer. a, Conidial tRNA esterified with ^3H -phenylalanine (—); and methylated with ^{14}C (---) in the presence of 1 mM 2',3'-CMP. b, Conidial tRNA esterified with ^3H -phenylalanine (—) and incubated in a methylation reaction mixture minus the methyl donor and minus CMP. c, Mycelial tRNA esterified with ^3H -phenylalanine (—); and methylated with ^{14}C (---), in the presence of 1 mM 2',3'-CMP. d, Mycelial tRNA esterified with ^3H -phenylalanine (—); and incubated in methylation reaction mixture minus the methyl donor and minus CMP.

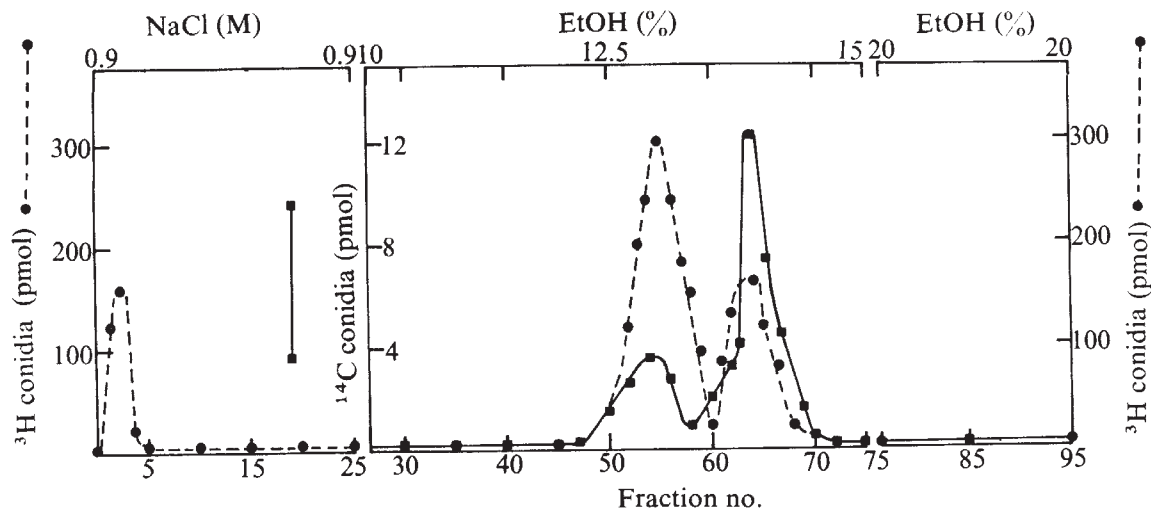


Fig. 3 Protection by methylation of phenylalanyl-tRNA of *N. crassa* from nuclease attack. Conidial tRNA^{Phe} esterified with ³H-phenylalanine and then methylated using a ¹⁴C-methyl donor (fractions 45–70 from Fig. 2a) were reincubated in the methylation reaction mixture but minus the 2',3'-CMP, then rechromatographed on the BD-cellulose column. ---, Phenylalanine-³H; —, methyl group-¹⁴C.

CMP. Since no nuclease products are evident, the free phenylalanine which appears early in the salt wash is due to deacylation rather than nuclease attack of the esterified tRNA^{Phe}.

Although methylation did not convert Phe-tRNA_{II}^c to Phe-tRNA_I^c, *in vitro*, the data from these four experiments indicate that methylation may protect under-methylated species of tRNA from nuclease attack. Whether methylation or 2',3'-CMP protected Phe-tRNA_{II}^c cannot be ascertained from the above experiments. To distinguish between the two possibilities, the following experiment was performed. The tRNAs in fractions 45–70 from Fig. 2a were collected, precipitated, and resuspended in the methylation reaction mixture but minus the 2',3'-CMP. The fractions were rechromatographed and the elution pattern is shown in Fig. 3. The ³H-phenylalanine appeared only as the free amino acid in the early portion of the salt wash and was not associated with nuclease products in the 20% ethanol wash. These data indicate that under-methylated Phe-tRNA_{II}^c was protected from nuclease attack by *in vitro* methylation. Thus, the rapid disappearance of Phe-tRNA_{II}^c observed *in vivo* may be the result of nuclease action on this labile species of Phe-tRNA.

In an earlier report⁶, we showed that phenylalanine exists as a unique NH₂-terminal amino acid in conidia. The transient Phe-tRNA of conidia might function in conidia-specific synthesis of these proteins. If so, a mechanism for its inactivation or destruction on conidial germination would seem to be an essential feature of regulation of this specific protein synthesis. The synthesis or maintenance of levels of glycoprotein associated with amino acid transport in conidia might be related to the transient Phe-tRNA. This glycoprotein is known to have phenylalanine as an NH₂-terminal amino acid (W. D. Stuart, personal communication) and amino acid transport mediated by this permease is known to decrease after germination of conidia. In conclusion, this transient tRNA offers a convenient handle for studying the operation of regulatory mechanisms involved in the transition from the conidial to the vegetative state of *Neurospora*.

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- 1 Borek, E., *Cancer Res.*, **31**, 591–721 (1971).
- 2 Gilliam, I., Millword, S., Blew, D., von Tigestrom, M., Winnor, E., and Tener, G. M., *Biochemistry*, **6**, 3043–3056 (1967).
- 3 Holley, R. W., Apgar, J., Doctor, B. P., Farrow, J., Marini, M., and Merrill, S., *J. biol. Chem.*, **236**(1), 200–202 (1961).
- 4 Hoskinson, R. M., and Khorana, H. G., *J. biol. Chem.*, **240**(5), 2129–2134 (1965).
- 5 Jervis, H. H., and DeBusk, A. G., *Neurospora Newsletter*, **20**, 21 (1973).
- 6 Reeves, R. H., Imura, N., Schwam, H., Weiss, G. B., Schulman, L., and Chambers, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 1450–1457 (1968).
- 7 Shearn, A., and Horowitz, N., *Biochemistry*, **8**(1), 295–303 (1969).
- 8 Shearn, A., and Horowitz, N., *Biochemistry*, **8**(1), 304–312 (1969).
- 9 Sueoka, N., and Kano-Sueoka, T., *Proc. natn. Acad. Sci. U.S.A.*, **52**, 1535–1540 (1964).
- 10 Sueoka, N., and Kano-Sueoka, T., *Progr. Nucleic Acid Res. molec. Biol.*, **10**, 23–55 (1970).
- 11 Wong, R. S. L., Scarborough, G. A., and Borek, E., *J. Bact.*, **108**(1), 446–450 (1971).

Deletion of the β -globin structure gene in hereditary persistence of foetal haemoglobin

HEREDITARY persistence of foetal haemoglobin (HPFH) is a condition characterised by the persistence of a high percentage of foetal haemoglobin into adulthood¹. The homozygous state has been described in the Negro^{2–4} and the Indian⁵; only haemoglobin F is present, and haemoglobins A and A₂ are absent. It has been suggested that in this syndrome γ -chain synthesis fails to switch to β -chain synthesis after birth. The reason for this failure is unknown, although deletions of the β - and δ -structural genes have been proposed⁶. We report here analysis of the DNA from a patient with the homozygous form of HPFH, using radioactive DNAs complementary to β - and γ -globin mRNA (β and γ cDNAs); the results are compatible with deletion of the β -globin structural locus.

The annealing of β - and γ cDNA to DNA is shown in Fig. 1. The β cDNA annealed to 65% with normal DNA, but only to 35% with HPFH DNA. In contrast, γ cDNA annealed equally to normal and HPFH DNA (90%).

In man, from the mode of inheritance of β -globin structural abnormalities, there is believed to be a single β structural locus⁷, while structural variation in the γ -globin chains demonstrates that there are two to four γ structural loci⁸. Analysis by DNA hybridisation has confirmed these gene numbers¹⁰. The smaller extent of hybridisation to the normal DNA of β cDNA compared with γ cDNA at the ratio cDNA:DNA used in these experiments is consistent with the larger number of γ -globin genes. The normal rate of hybridisation of γ cDNA to both DNAs suggests that the γ -globin genes in HPFH are intact. This agrees with the finding that the foetal haemoglobin of this patient contains both the " γ and the $\Lambda\gamma$ types". The significantly decreased

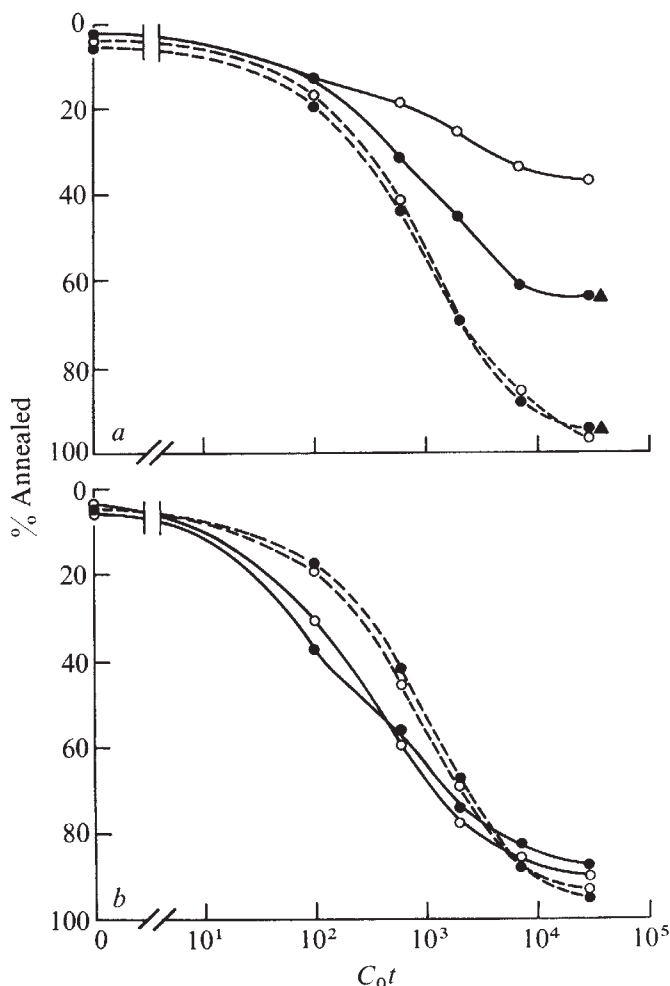


Fig. 1 Rate of annealing of normal and HPFH DNA. DNA was prepared from fibroblasts cultured from a skin biopsy of an HPFH patient, and from the skin fibroblasts and white blood cells (WBC) of two non-thalassaemic controls, as before⁷. β cDNA was transcribed from the 10S RNA ($\alpha : \beta = 1 : 8$ by translation) from the reticulocytes of a patient with haemoglobin H disease, and γ cDNA was transcribed from the reticulocyte RNA ($\alpha : \beta : \gamma = 0 : 1 : 8$) of a patient with hydrops fetalis. ^{32}P - α -dCTP was used as the label, and the methods for synthesis and isolation of the cDNA were as previously described⁷. Both cDNAs were used without further enrichment of the β or γ sequences. Each 10- μl reaction mixture contained 400 c.p.m. (3.7 pg) of ^{32}P -labelled β or γ cDNA, 75 μg of cellular DNA, 500 c.p.m. of ^3H -dCTP labelled HeLa unique sequence DNA as internal controls, 0.5 M NaCl, 0.04 M Tris-HCl (pH 7.4) and 1.5 mM EDTA, and was incubated for 0–72 h at 68 °C. Hybrids were assayed with hydroxylapatite⁷. a, β cDNA; b, γ cDNA. ●, Normal WBC DNA; ○, HPFH fibroblast DNA; —, cDNA; ---, unique sequence HeLa DNA; ▲, shows that the annealing of β cDNA to normal skin fibroblast DNAs was identical to the WBC DNA.

hybridisation of β cDNA in HPFH is compatible with the deletion of all or part of the single β locus. There are several possible reasons why some β cDNA annealed to the HPFH DNA. First, 10–15% can be attributed to the α sequence known to be present in the β cDNA. Second, some portion of the β -structural gene may remain undeleted. Third, nonspecific α cDNA hybrids with low thermal stability have been found in hydrops fetalis where the α -globin structural genes are deleted (ref. 7 and unpublished work of Y.W.K.). Similar nonspecific hybridisation of the β cDNA may occur in the absence of β -globin structural genes.

We cannot infer from these data whether or not the δ -globin gene is affected, as the extent of cross hybridisation between β and δ sequences is not known. The absence of haemoglobin A₂ in homozygous HPFH suggests involve-

ment of the δ -globin gene also, but this may not necessarily be due to deletion, as other mechanisms resulting in the absence of δ -chain synthesis may be possible.

In conclusion, the decreased hybridisation of the β cDNA to HPFH DNA indicates that all or a large portion of the β -globin locus is deleted in this syndrome.

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- Edington, G. M., and Lehmann, H., *Br. Med. J.*, **1**, 1308–1311 (1955); *ibid.*, **2**, 1328–1330 (1955).
- Wheeler, J. T., and Krevans, J. R., *Bull. Johns Hopkins Hosp.*, **109**, 217–233 (1961).
- Ringelmann, B., Konotey-Ahulu, F. I. D., Lehmann, H., and Lorkin, P. A., *Acta Haemat.*, **43**, 100–110 (1970).
- Siegel, W., et al., *Ann. Int. Med.*, **72**, 533–536 (1970).
- Sukumaran, P. K., et al., *Br. J. Haemat.*, **23**, 403–417 (1972).
- Herman, E. C., Jr, and Conley, C. L., *Am. J. Med.*, **29**, 9–17 (1960).
- Taylor, J. M., et al., *Nature*, **251**, 392–393 (1974).
- Rucknagel, D. L., and Winter, W. P., *Ann. N.Y. Acad. Sci.*, **241**, 80–92 (1974).
- Huisman, T. H. J., et al., *New Engl. J. Med.*, **285**, 711–715 (1971).
- Ottolenghi, S., et al., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2294–2299 (1975).

Effect of DTNB light chain on the interaction of vertebrate skeletal myosin with actin

MYOSIN from vertebrate skeletal muscles contains four low molecular weight subunits which are thought to play a functional role in the enzymic activity of myosin^{1,2}. Removal of 'alkali light chains' (21,000 and 17,000 g mol⁻¹) results in complete loss of ATPase activity, but dissociation of 'DTNB light chain' (18,000 g mol⁻¹) leaves the enzymic activity of myosin essentially unaffected^{3–5}. Since harsh solvents are required to dissociate alkali light chains from heavy chains (as the name implies), it is unclear whether the activity loss is related primarily to denaturation of the heavy chain or to removal of the light chain⁷.

The only light chain for which a function has been clearly defined is that associated with myosin from molluscan muscles. It is now well documented that calcium regulation in molluscs is through the thick filament; the thin filaments lack troponin and do not bind calcium⁸. Thus the 'EDTA light chain' of scallop myosin is a true regulatory subunit in that it controls whether the muscle is in an active or a resting state⁹. No comparable calcium sensitivity has been shown for vertebrate skeletal myosin, although rabbit myosin¹⁰, as well as isolated DTNB light chains¹¹, can bind calcium in the 10⁻⁵ M range. Nevertheless, some effects of calcium on the properties of myosin filaments have been observed. The sedimentation coefficient of isolated, native thick filaments increases by about 3% with increasing calcium levels¹¹. X-ray diffraction patterns from contracting muscle indicate a movement of cross bridges on activation, which is independent of the presence of actin¹². Both studies suggest a conformational change occurring within the thick filament. The biological significance of these observations however, is unclear; and an involvement of the DTNB light chain in these structural changes has not been unequivocally

demonstrated. We present here data showing that the DTNB light chain is directly involved in the interaction between myosin and actin, and moreover, that the strength of this interaction depends on the concentration of calcium present in the medium.

At some stage in the contractile cycle, myosin is probably bound to actin in a 'rigor'-type linkage. The energy needed to break this bond is derived from the binding of ATP to myosin. A value for the association of myosin and actin in the absence of ATP has been estimated to be no less than 10^6 M^{-1} and may be considerably higher^{13,14}. We have redetermined this parameter by analysing the binding of heavy meromyosin (HMM) and heavy meromyosin subfragment 1 (S1) to F actin in the analytical ultracentrifuge, using the scanning absorption optical system at 230 nm. By adding an increasing concentration of enzyme to a fixed amount of F actin and sedimenting the complex, the free concentration of enzyme can be measured in the supernatant and a binding isotherm constructed. The details of these experiments will be presented elsewhere, but the principal conclusions may be summarised as follows: the association constant for HMM and actin is $1 \times 10^6 - 4 \times 10^6 \text{ M}^{-1}$ in 0.1 M KCl, 10 mM imidazole, pH 7.0, 1 mM MgCl_2 , 5 mM Pi; the corresponding value for S1 is about 10^5 M^{-1} . Unexpectedly, we found that these values increased nearly tenfold on addition of 0.3 mM EGTA to the basic solvent, whereas 0.1 mM Ca^{2+} caused a significant reduction in binding¹⁵. Figure 1 shows that the midpoint of this transition in binding occurs at about $p\text{Ca}$ 5. Although this amount of calcium is an order of magnitude higher than that required to activate skeletal muscles, it is still well within the physiological range of calcium reached in a contracting muscle cell.

Having shown a calcium response in vertebrate skeletal myosin, the question arises as to the site of calcium action. Since calcium affects the interaction with actin, an involvement of the head region of myosin is indicated; and, indeed, both HMM and S1 are sensitive to calcium. The magnitude

Fig. 1 Effect of calcium concentration on the extent of actomyosin formation. Rabbit myosin (1.27 mg ml^{-1}) was mixed with F actin (0.24 mg ml^{-1}) in 0.34 M KCl, 10 mM imidazole (pH 7.5), 5 mM KPi and 1 mM MgCl_2 in a volume of 2 ml. The calcium level was adjusted by Ca-EGTA-EGTA buffers. The reaction mixture was stirred for 30 min in the cold, before centrifugation at 40,000 r.p.m. in the analytical ultracentrifuge. The concentration of unbound myosin in the supernatant was determined at 280 nm with the photoelectric scanner (Model E). Each point represents the difference between the absorbance (A_{Ca}) in the presence of calcium and in its absence (A_{EGTA}), relative to the absorbance in 0.3 mM EGTA. $p\text{Ca}$ is the negative logarithm of the molar concentration of free calcium. Five measurements could be made simultaneously using the AN-G rotor with the multiplexer accessory. $\circ$, $\bullet$, $\blacktriangle$, Different preparations.

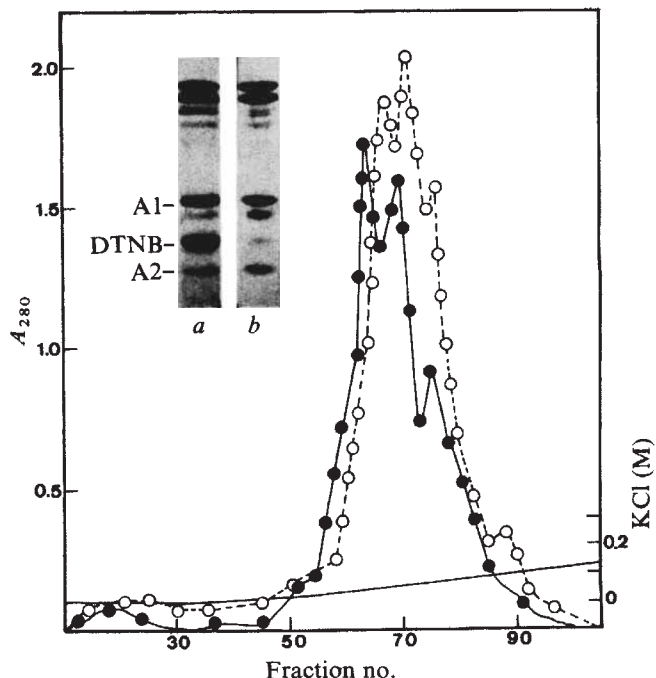
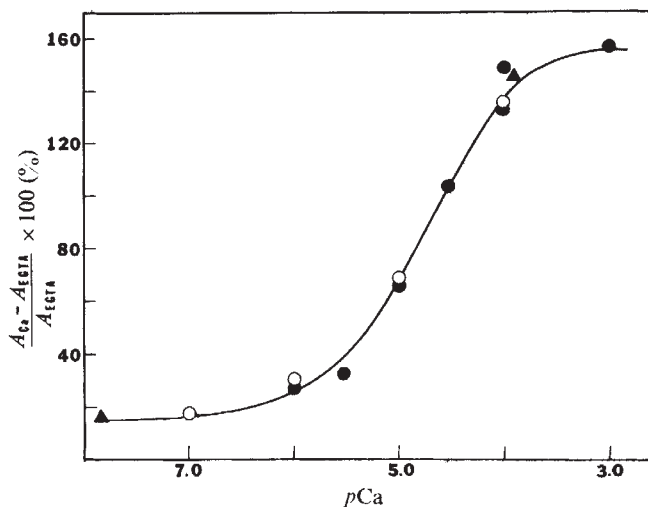


Fig. 2 Chromatographic purification of subfragment 1. Rabbit myosin at about 2% was dialysed against 0.2 M ammonium acetate, pH 7.2, in the presence of either 2 mM MgCl_2 or 2 mM EDTA. The precipitated myosin was digested at room temperature for 7 min with 0.03 mg ml^{-1} papain (Worthington). Digestion was stopped by adding iodoacetic acid to a final concentration of 1.0 mM. Insoluble material was removed by centrifugation and the supernatant dialysed against 0.05 M Tris-HCl (pH 7.9), 0.5 mM dithiothreitol, containing either 2 mM MgCl_2 or 2 mM EDTA. About 450 mg subfragment 1 was applied to a $2.5 \times 60 \text{ cm}$ DEAE-cellulose column (Whatman DE52) equilibrated in the same buffer. The protein was eluted with a linear KCl gradient (0.0–0.5 M KCl in a total volume of 2 l) at a flow rate of 45 ml h^{-1} , collecting 6 ml per tube. Fractions 60–70 of Mg-S1 ($\bullet$) and 60–72 of EDTA-S1 ($\circ$) were pooled and the material salting out between 49 and 58% saturated ammonium sulphate retained for further experiments. Representative samples of Mg-S1 (a) and EDTA-S1 (b) at $15\text{-}\mu\text{g}$ loads on 10% SDS-polyacrylamide gels are shown above.

of the response, however, varied from one preparation to the next, suggesting possible proteolytic degradation of essential sites. This variation was most pronounced for S1, where some preparations gave less than 20% of the effect observed with myosin.

The standard procedure for preparing S1, the 'single head' of myosin, is to digest a suspension of myosin with a low concentration of papain¹⁴. S1 prepared in this manner shows one symmetrical peak in the ultracentrifuge, has a high level of ATPase activity, and is regulated by the troponin-tropomyosin system. Gel electrophoresis of rabbit S1 gives two bands of about 90,000 and three light chain components plus an extra band (which probably originates from cleavage of the heavy chain)¹⁶ in the $20,000 \text{ g mol}^{-1}$ region. Densitometry of such gel patterns shows that less DTNB light chain is present in S1 than occurs per head of native myosin, a finding consistent with radiochemical studies indicating a loss of $\sim 0.5 \text{ mol DTNB light chain per mol S1}$ (ref. 3).

The susceptibility of DTNB light chain to proteolysis is reminiscent of the early difficulties in preparing S1 from scallop myosin⁹. Depending on whether divalent cations were present or absent in the digestion medium, EDTA light chain remained intact or was completely degraded by papain. These results suggested that perhaps the stability of DTNB light chain might be similarly affected by the presence of magnesium ions. Consequently the procedure for digesting rabbit myosin to S1 was modified to include 2 mM MgCl_2 or 2 mM EDTA in all solutions (see legend to

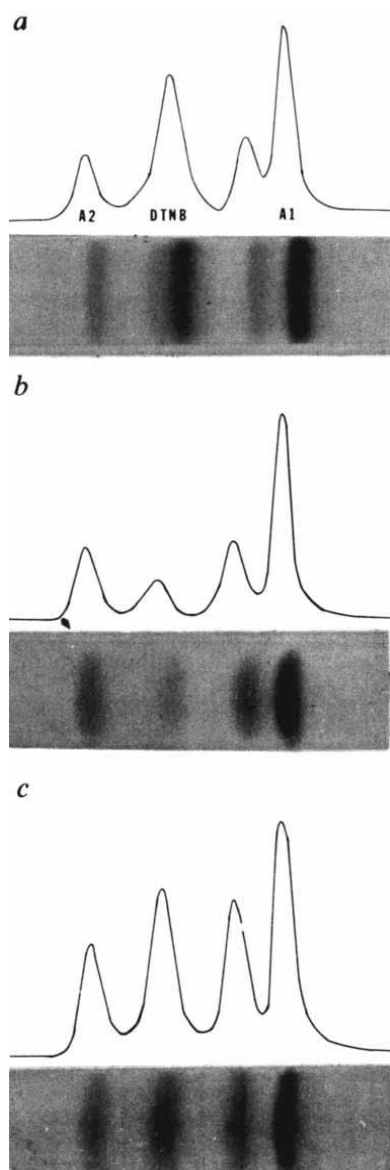


Fig. 3 Relative amounts of light chain in subfragment 1 preparations. Samples of proteins, at loads determined to be within the linear range of dye uptake, were run on 10% SDS-polyacrylamide gels as described previously⁴. Gels were fixed and stained in 25% 2-propanol, 10% acetic acid, containing 0.025% Coomassie brilliant blue; destaining was by diffusion in 10% acetic acid¹⁸. Gels were scanned on a Joyce-Loebl microdensitometer, and areas determined from the scans with a planimeter. *a*, 14 μ g Mg-S1; *b*, 14 μ g EDTA-S1; *c*, 18 μ g EDTA-S1 recombined with DTNB light chain.

Fig. 2 for details). Chromatographic profiles of Mg-S1 and EDTA-S1 are illustrated in Fig. 2, together with electrophoretic patterns of the pooled fractions. These gels provide clear evidence that S1 prepared in the presence of magnesium ions has a significantly higher proportion of DTNB light chain than S1 digested in EDTA. In fact, densitometry of the chromatographed samples showed that Mg-S1 has approximately the same content of DTNB light chain as myosin (Fig. 3 and Table 1). The relative amount of alkali 1 to alkali 2 was fairly constant in both types of subfragment, and gave a ratio similar to that found for native rabbit myosin¹⁷.

A preliminary characterisation of Mg-S1 and EDTA-S1 revealed few major differences in their properties: Both appeared monodisperse in the ultracentrifuge and sedimented at about equal rates, although inspection of the shape of the sedimentation velocity profile suggested a

Table 1 Relative amounts of light chain in subfragment 1

Subfragment	Experiment	Alkali 1	DTNB light chain	Alkali 2
EDTA-S1	1	1.15	0.29	0.85
	2	1.29	0.36	0.71
	3	1.14	0.48	0.86
Mg-S1	1	1.28	1.79	0.72
	2	1.21	1.71	0.79
	3	1.71	2.00	0.29
Recombined	1	1.23	0.66	0.77
	2	1.20	1.12	0.80
	3	1.03	0.81	0.97

Amount of each light chain relative to the total light chain content was determined by dividing the densitometer area by the molecular weight and normalising values to mol ($A1 + A2 = 2$). Each value represents an average of 4–6 determinations. Conditions for electrophoresis were as in Fig. 3. "Recombined" subfragment was prepared by adding total light chains (300 mg) or purified DTNB light chain¹⁸ (80 mg) to EDTA-S1 (300 mg), and separating the subfragment from the uncombined light chains by ion-exchange chromatography as in Fig. 2.

possible difference in frictional coefficients. The Ca^{2+} - and EDTA-activated ATPases for the two proteins were in the range of 3 and 10 s^{-1} , respectively; and the actin-activated ATPase was between 13 and 16 s^{-1} . The activity of both preparations was inhibited by troponin-tropomyosin to the extent of 70–80% in the absence of calcium. These results confirm previous reports that DTNB light chain is not involved in actin-linked regulation¹⁸.

The most significant difference between the preparations of Mg-S1 and EDTA-S1 was in their response to calcium,

Fig. 4 Ultracentrifuge scanner traces of the binding of subfragment 1 to F actin as a function of calcium. Mg-S1 (0.33 $mg\ ml^{-1}$) or EDTA-S1 (0.33 $mg\ ml^{-1}$) in 0.10 M KCl, 10 mM imidazole (pH 7.0), 5 mM KPi, 1 mM $MgCl_2$, containing either 0.1 mM $CaCl_2$ or 0.3 mM EGTA, was added to F actin (0.24 $mg\ ml^{-1}$) in a final volume of 2 ml. The mixture was stirred for 30 min before centrifugation at 40,000 r.p.m. The concentration of free enzyme remaining in the supernatant was determined at 230 nm from the magnitude of the plateau region, and corrected for unpolymersed actin. *a* and *b*, Mg-S1 in the presence and absence of calcium, respectively. *c*, EDTA-S1; scan was the same for calcium or EGTA.

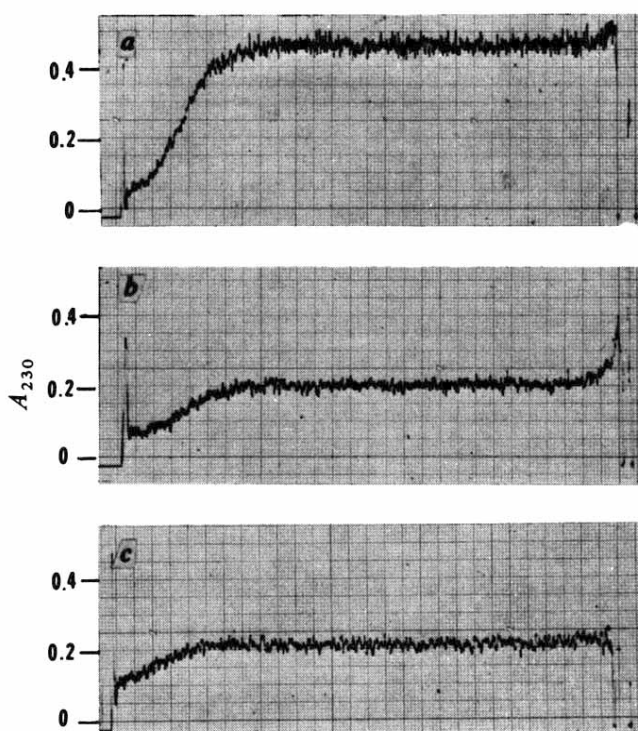


Table 2 Effect of calcium on binding of subfragment 1 to actin

Subfragment	Experiment	0.1 mM CaCl ₂	A_{230} 0.3 mM EGTA	Calcium sensitivity (%)
EDTA-S1	1	0.28	0.28	—
	2	0.37	0.36	—
	3	0.24	0.24	—
Mg-S1	1	0.76	0.31	147
	2	0.78	0.32	148
	3	0.46	0.20	130
Recombined	1	0.56	0.28	100
	2	0.58	0.31	87
	3	0.45	0.32	41

Absorbances for subfragments were obtained as in Fig. 4. "Calcium sensitivity" is expressed as $((A_{Ca} - A_{EGTA})/A_{EGTA}) \times 100$.

using the binding of S1 to actin as an assay. Figure 4 shows that when Mg-S1 was reacted with actin in the presence of calcium, twice as much enzyme remained unbound in the supernatant as when a chelating agent was present. In contrast, the amount of free EDTA-S1 remained invariant with calcium concentration. Several such experiments are summarised in Table 2. Although the difference in DTNB light chain content is the most striking feature of the S1 preparations, it must be recognised that minor modifications in the heavy chain could be responsible for the unresponsiveness of EDTA-S1 to calcium. It was therefore important to demonstrate that addition of DTNB light chain to EDTA-S1 would restore the calcium effect. Preliminary efforts at recombination are shown in Tables 1 and 2 and Fig. 3. In spite of difficulties in achieving full recombination of DTNB light chain, the results do indicate a partial restoration of the calcium response.

The experiments reported here demonstrate that the DTNB light chain is directly involved in the binding of calcium to vertebrate skeletal myosin. These findings should not be interpreted to mean that a second control mechanism necessarily exists in vertebrate muscles. The effect of calcium observed here is to weaken rather than to strengthen the actomyosin interaction. Furthermore, the level of calcium required for this effect is considerably higher than that needed to activate muscle. Nevertheless, the results show that the properties of the myosin head can be affected by the DTNB light chain, and perhaps it is not too unrealistic to speculate that subunit interactions of this kind may have an important role in the attachment of a cross bridge to actin during the contractile cycle.

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- Stracher, A., *Biochem. biophys. Res. Commun.*, **35**, 519–525 (1969).
- Dreizen, P., and Gershman, L. C., *Biochemistry*, **9**, 1688–1693 (1970).
- Weeds, A. G., and Lowey, S., *J. molec. Biol.*, **61**, 701–725 (1971).
- Lowey, S., and Risby, D., *Nature*, **234**, 81–85 (1971).
- Gazith, J., Himmelfarb, S., and Harrington, W. F., *J. biol. Chem.*, **245**, 15–22 (1970).
- Weeds, A. G., *Nature*, **223**, 1362–1364 (1969).
- Leger, J. J., and Marotte, F., *FEBS Lett.*, **52**, 17–21 (1975).
- Kendrick-Jones, J., Lehman, W., and Szent-Gyorgyi, A. G., *J. molec. Biol.*, **54**, 313–326 (1970).
- Szent-Gyorgyi, A. G., Szentkiralyi, E. M., and Kendrick-Jones, J., *J. molec. Biol.*, **74**, 179–203 (1973).
- Bremel, R. D., and Weber, A., *Biochim. biophys. Acta*, **376**, 366–374 (1975).
- Morimoto, K., and Harrington, W. F., *J. molec. Biol.*, **88**, 693–709 (1974).
- Haselgrove, H. C., *J. molec. Biol.*, **92**, 113–143 (1975).

- Eisenberg, E., Dobkin, L., and Kielley, W. W., *Biochemistry*, **11**, 4657–4660 (1972).
- Margossian, S. S., and Lowey, S., *J. molec. Biol.*, **74**, 313–330 (1973).
- Margossian, S. S., and Lowey, S., *Fedn Proc.*, **34**, 671 (1975).
- Stone, D., and Perry, S. V., *Biochem. J.*, **131**, 127–137 (1973).
- Sarker, S., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 14–17 (1972).
- Holt, J. C., and Lowey, S., *Biochemistry* (in the press).

Fluorescence lifetimes in the photosynthetic unit

THE fluorescence intensity of a photosynthetic organism at any instant is a measure of the concentration of excited chlorophyll and it has, therefore, been one of the most studied of all the phenomena related to photosynthesis. Fluorescence lifetimes are a direct measure of the rate of energy transfer from the light collecting unit to the chemical trap. Lifetimes in the nanosecond region have been measured by many workers, following the pioneering work of Brody and Rabinowitch¹, but these have been near the limit of time resolution of the apparatus used. Much shorter average lifetimes have been measured by phase shift methods². The picosecond pulses which are available from mode-locked lasers have now made possible a fully time-resolved study of fluorescence decay in the photosynthetic units of algal chloroplasts and their fragments.

The first laser pulse measurements on picosecond fluorescence lifetimes used the electro-optical shutter^{3–5} but the irreproducibility of pulse intensity makes this a rather imprecise method. The streak camera^{6,7} has greatly improved the precision but much of this is lost if photographic recording is used. With silicon-target, vidicon multichannel detection, which we have used in this work, the streak camera provides the best precision available at present for picosecond fluorescent kinetic studies. The picosecond pulses were generated from a mode-locked Nd³⁺ glass oscillator and the second harmonic at 530 nm was produced with 10–15% efficiency by a temperature-tuned caesium dihydrogen arsenate crystal. The train of pulses (approximately 100 with pulse separation of 6.7 ns) was directed at 45° on to the front surface of a cuvette (1 mm thick) containing the sample. The fluorescence passed through a Barr and Stroud DB5 CYAN filter to the slit of an Imacon streak camera which had an S20 photocathode. The fluorescence from one pulse near the centre of the train was selected for examination; this pulse had a duration 5–10 ps and its energy was typically 200 μJ. The signal was recorded on a 500-channel optical multichannel analyser and was calibrated both in intensity and in time.

Fluorescence lifetimes of algae, chloroplasts and chloroplast fragments have been measured. The algae used were *Chlorella pyrenoidosa* and *Porphyridium cruentum* grown in liquid culture⁸ and collected during the logarithmic growth phase. Chloroplasts were isolated from spinach leaves (*Spinacea oleracea*) by a method previously reported⁹ except they were subjected to osmotic shock before being suspended in the experimental medium which consisted of 0.33 M Sorbitol, 1 mM MgCl₂, 1 mM MnCl₂, 2 mM EDTA and 50 mM HEPES brought to pH 7.6 with KOH. Subchloroplast particles were prepared by the method of Anderson and Boardman¹⁰. Measurements with algae were made with 10–15% suspensions, and with chloroplasts and subchloroplast particles the chlorophyll, as determined by Arnon's method¹¹, was about 3 mg ml⁻¹. Fluorescence emission and yields were also measured in the nanosecond region of the decay using a conventional single-photon counting system.

Figure 1 shows the fluorescence decay curves for the two algae and Fig. 2 shows the decays for untreated spinach chloroplasts and for the same chloroplasts treated with 20 mM sodium dithionite, which reduces the activity of the chemical traps. The decays of *Chlorella*, *Porphyridium* and untreated chloroplasts were exponential from 20 to 300 ps after excitation and their decay times are given in Table 1. Beyond 300 ps there

Table 1 Fluorescence lifetimes of algae and chloroplasts

	τ (ps)
<i>Chlorella</i>	108 ± 10
<i>Porphyridium</i>	92 ± 10
Chloroplasts	134 ± 10
Chloroplasts + dithionite	130 ± 20
	$1,450 \pm 150$

is evidence of a very weak longer lived component of the fluorescence.

Dithionite treatment of chloroplasts had little effect on the main component of fluorescence but markedly increased the intensity and lifetime of the longer lived component and the total yield of fluorescence. The longer lived component was measured on our nanosecond single-photon counting apparatus (1.6 ns) as well as on the picosecond apparatus (1.45 ns) and the decay was exponential. These lifetimes agree with previous measurements in this region¹².

Sub-chloroplast particles centrifuged at 1,000, 10,000, 50,000 and 144,000g gave increasing intensity of the shorter lived component over that of the longer lived one. This is probably to be attributed to the higher proportion of photosystem I (PS I) in the high *g* centrifuged particles. This, together with the effect of dithionite on the longer lived component of the chloroplast, suggests that the shorter and longer decays are to be identified with PS I and PS II respectively.

In the chloroplasts treated with dithionite, the two fluorescence decays (attributed to PS I and PS II) are quite distinct and

Fig. 1 Fluorescence decay of intact algae. *a*, *Porphyridium cruentum*; *b*, *Chlorella pyrenoidosa*.

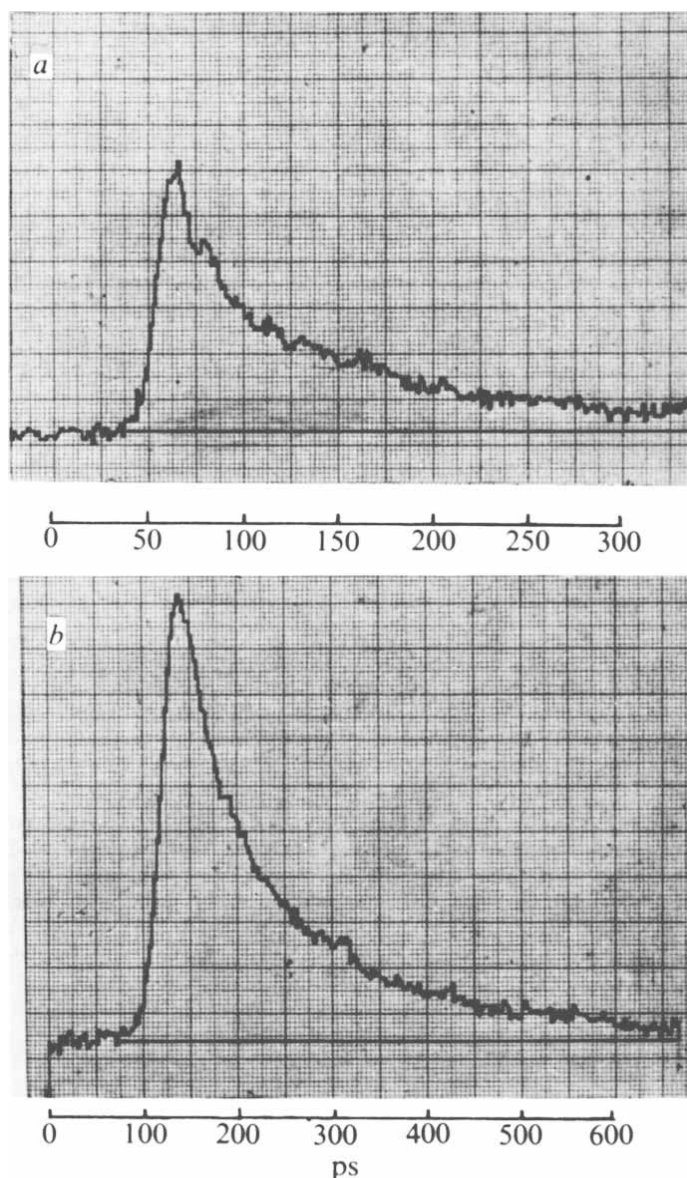
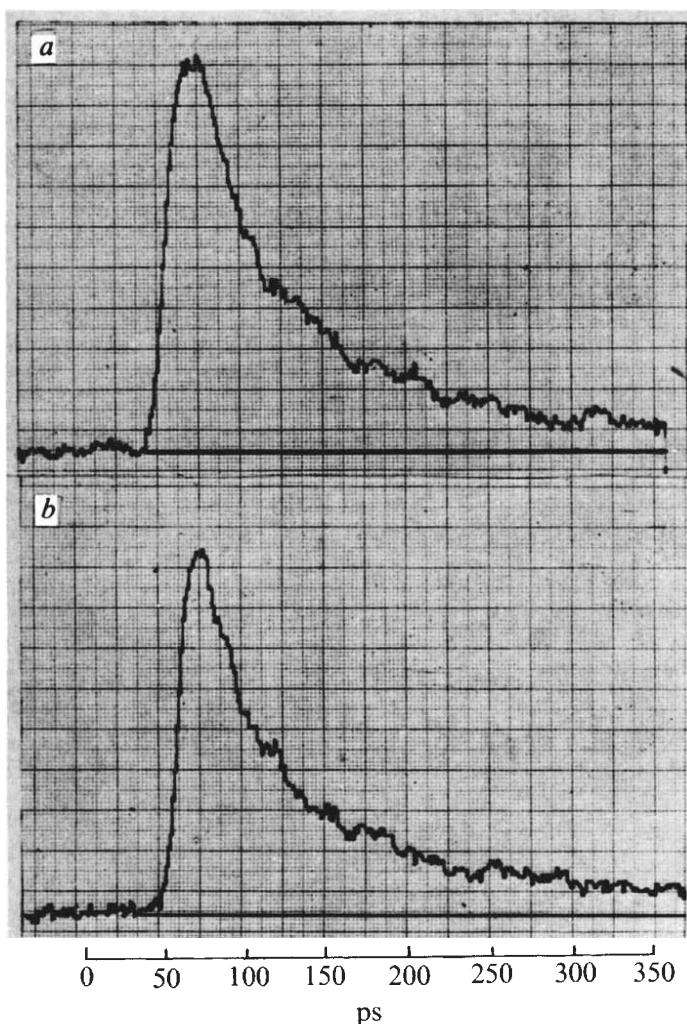


Fig. 2 Fluorescence decay of chloroplasts. *a*, Spinach chloroplast; *b*, spinach chloroplast and dithionite.

it has been possible to make quantitative measurements on their relative contributions to the fluorescence. The relative numbers, f , of chlorophyll *a* molecules initially excited in the two systems is given directly by the ratio of initial fluorescence intensities of the two components, which is 4.5, the shortest component being the more intense. From this, and the measured lifetimes, the fluorescence yield of each system may be calculated from the relationships:

$$\phi_I = f_I(\tau_I/\tau_0)$$

$$\phi_{II} = f_{II}(\tau_{II}/\tau_0)$$

where ϕ , τ and f are the fluorescence yield, the lifetime and the fraction of excitation transmitted to chlorophyll *a* molecules of

Table 2 Fluorescence yields of dithionite-treated chloroplasts

	τ (ps)	f	ϕ (calculated)	(measured) ϕ
PS I	130	0.82	0.0056	0.020
PS II	1,450	0.18	0.014	
				0.024

photosystem I, and subscript II refers to the same quantities for system II; τ_0 is 19 ns, the natural lifetime of chlorophyll *a* (ref. 13). It will be seen from Table 2 that the calculated yield of total fluorescence agrees well with our direct measurements for the same chloroplasts.

It should be noted that the ratio, *f*, of 4.5, determined from the initial fluorescence intensities of the two decays, involves the accessory pigment absorption and measures the ratio of chlorophyll molecules excited in each system rather than the ratio of unexcited chlorophyll molecules. It refers to the excitation wavelength of 530 nm. The ratio is higher than has been predicted from measurements of the quantum yield of photosynthesis at this wavelength^{14,15} and casts doubt on the feasibility of distinguishing photosystems I and II on the basis of their lifetimes.

Kollman, Shapiro and Campillo⁷ have attributed the short lifetimes of chlorophyll fluorescence *in vivo* to concentration quenching, on the grounds that similar lifetimes are found in concentrated solutions of chlorophyll. Such an interpretation would be inconsistent with the operation of efficient photosynthesis and, although concentration quenching, probably of a special type which we discuss elsewhere, accounts for the lifetimes of fluorescence in concentrated solutions of chlorophyll *in vitro*, it cannot be important in an efficient photosynthetic system. We have considered one other possible cause of quenching of excited chlorophyll molecules in the *in vivo* measurements reported here—the intensity during measurement is so high that a significant fraction of the chlorophyll molecules is excited. Singlet-singlet annihilation could become important in these conditions, and possibly singlet-triplet annihilation involving triplets formed in earlier pulses. We therefore carried out the measurements on porphyrin over a tenfold range of intensities; the decay rates of fluorescence were unaffected by the intensity change and we conclude that exciton interactions are unimportant in the conditions of our experiments. In some cases, the initial rate, immediately after the flash, was somewhat faster than that reported in Table 1 but this would be expected from the time-dependent term in the theory of excitation diffusion (see ref. 16).

The lifetimes we have recorded are a direct measure of the average time taken for an absorbed photon to be trapped at the reaction centre in the conditions of our experiments.

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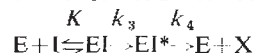
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- 1 Brody, S. S., and Rabinowitch, E., *Science*, **125**, 555–558 (1957).
- 2 Borisov, A. Yu., and Il'ina, M. D., *Biochim. biophys. Acta*, **305**, 364–371 (1973).
- 3 Duguay, M. A., and Hansen, J. W., *Appl. Phys. Lett.*, **15**, 192–194 (1969).
- 4 Seibert, M., and Alfano, R. R., *Biophys. J.*, **14**, 269–283 (1974).
- 5 Porter, G., Reid, E. S., and Tredwell, C. J., *Chem. Phys. Lett.*, **29**, 469–472 (1974).
- 6 Bradley, D. J., Liddy, B., and Sleat, W. E., *Optics Commun.*, **2**, 391–395 (1971).
- 7 Kollman, V. H., Shapiro, S. L., and Campillo, A. J., *Biochem. biophys. Res. Commun.*, **63**, 917–923 (1975).
- 8 Shieh, Y. J., and Barber, J., *Biochim. biophys. Acta*, **233**, 594–603 (1971).
- 9 Barber, J., Telfer, A., and Nicolson, J., *Biochim. biophys. Acta*, **357**, 161–165 (1974).
- 10 Anderson, J. M., and Boardman, N. K., *Biochim. biophys. Acta*, **112**, 403–421 (1966).
- 11 Arnon, D. I., *Pl. Physiol.*, **24**, 1–15 (1949).
- 12 Briantais, J. M., Merkelo, H., and Govindjee, *Photosynthetica*, **6**, 133–141 (1972).
- 13 Beddard, G. S., Porter, G., and Weese, G. M., *Proc. R. Soc. A*, **342**, 317–325 (1975).
- 14 Emerson, R., and Lewis, C. M., *Am. J. Bot.*, **30**, 165–178 (1943).
- 15 Williams, W. P., Murty, N. R., and Rabinowitch, E., *Photochem. Photobiol.*, **9**, 455–469 (1969).
- 16 Noyes, R. M., in *Progress in Reaction Kinetics*, **1** (edit. by Porter, G.), 129–160 (Pergamon, Oxford, 1961).

Fragmentation of benzylpenicillin after interaction with the exocellular DD-carboxypeptidase-transpeptidases of *Streptomyces* R61 and R39

THE killing target of penicillin in bacteria is a membrane-bound transpeptidase which catalyses peptide cross linking during wall peptidoglycan synthesis^{1,2}. *Streptomyces* R61 and R39 excrete during growth DD-carboxypeptidase-transpeptidase enzymes^{3,4} which seem to be soluble forms of the corresponding membrane-bound transpeptidases⁵. The exocellular enzymes (E) convert penicillin (I) in to a chemically altered and biologically inactive compound (X)^{6,7}. Kinetically, the simplest mechanism⁵ for the conversion of I into X is



The first step, a rapid equilibrium process, leads to the formation of an equimolar and inactive enzyme-antibiotic complex EI. This complex isomerises into a modified complex EI* which, in turn, undergoes irreversible breakdown. If the experiment is carried out in conditions in which the enzyme is stable, the enzyme is reactivated and recovers its initial penicillin sensitivity. The breakdown of complex EI* is a slow process. At 37 °C and in 10 mM Na phosphate buffer, pH 7.0 (in which conditions the R61 enzyme is stable) the half life of the R61 enzyme-benzylpenicillin EI* complex is 80 min (ref. 7). At 37 °C and in 0.1 M Tris-HCl buffer, pH 7.7, containing 0.1 M NaCl and 0.05 M MgCl₂ (in which conditions the R39 enzyme is stable), the half life of the R39 enzyme-benzylpenicillin is 4,250 min (ref. 6). As the enzyme is reactivated during the process, no enzyme is irretrievably lost on reaction with penicillin and after several cycles of inactivation and reactivation, both the enzyme and the accumulated X

Fig. 1 Mass spectra of authentic phenylacetylglutamine (A, molecular weight = 193) and X compound (B). ¹⁴C-X (0.5 µeq) was dissolved in 100 µl of 6 N HCl and extracted, at room temperature, with 300 µl of ethyl acetate (yield of the extraction 60% in terms of d.p.m.). Mass spectrography was carried out on a sample of the extract containing 5 neq of ¹⁴C-X and compared with that given by 1 µg of phenylacetylglutamine. Spectra were recorded at 70 eV with an ionisation current of 60 µA. The sample was directly introduced (193: molecular ion M⁺).

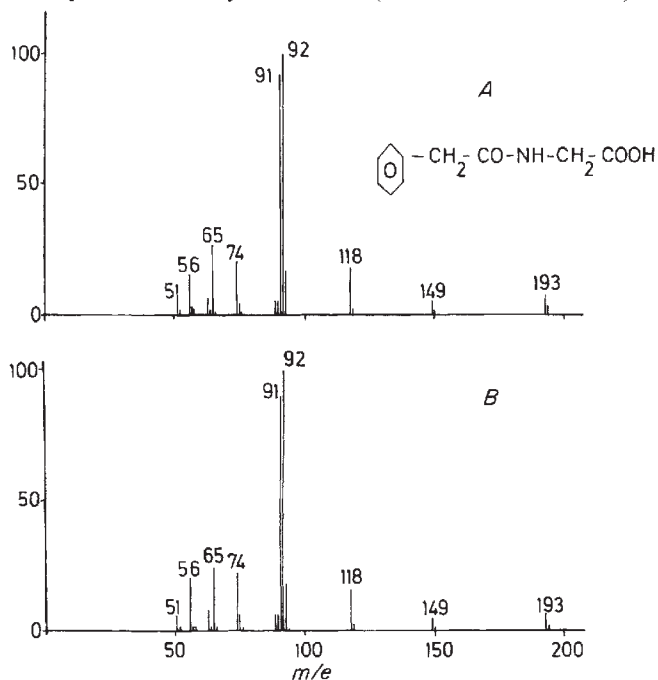


Table 1 R_f values of ^{14}C -X and phenylacetyl-glycine and of HCl-hydrolysed ^{14}C -X and phenylacetic acid, by cochromatography on Silica-Gel G thin-layer plates

Enzyme used for the preparation of ^{14}C -X	Solvent	R_f values of			
		^{14}C -X	Added phenylacetyl-glycine	HCl-hydrolysed ^{14}C -X	Added phenylacetic acid
R61	a	0.77	0.76	0.80	0.80
	b	0.71	0.70		
	c	0.40	0.40	0.56	0.55
R39	a	0.78	0.77		
	b	0.71	0.69		
	c	0.36	0.36		

Solvents: a, 1-butanol– H_2O –acetic acid–ethanol: 10:4:3:3 (v:v:v:v); b, water–1-butanol–acetic acid: 50:10:10 (v:v:v) upper phase; c, chloroform–methanol–acetic acid: 88:10:2 (v:v:v).

Note that the R_f values of benzylpenicilloic acid are 0.65 in solvent a and 0.55 in solvent b. Benzylpenicilloic acid is immobile in solvent c.

compound can be reisolated. This technique was applied to ^{14}C -benzylpenicillin (benzyl labelled) and the ^{14}C -X compound produced was isolated.

A solution of R61 enzyme (0.9 μmol ; molecular weight 38,000) in 20 ml of phosphate buffer was supplemented at 37 °C with 0.72 μmol of ^{14}C -benzylpenicillin (0.7 mCi mmol^{-1}). Every 30 min, an additional 0.144 μmol of ^{14}C -benzylpenicillin was added until a total amount of 29 μmol of antibiotic was used. Filtration on Sephadex G-25 and elimination of phosphate by addition of acetone at 0 °C yielded 19 μeq of purified ^{14}C -X. Similarly, a solution of R39 enzyme (11.7 nmol; molecular weight 53,000) in 100 μl of Tris–NaCl– MgCl_2 buffer was supplemented with 14.5 nmol of ^{14}C -benzylpenicillin (25 mCi mmol^{-1}) and the mixture was incubated for 51 h at 37 °C. Filtration on Sephadex G-25 yielded 4 neq of ^{14}C -X.

The ^{14}C -X obtained with the R61 enzyme was identified as ^{14}C -phenylacetyl-glycine on the following grounds. (1) Acid hydrolysis with 6 N HCl at 120 °C yielded glycine (yield 100% with the amino acid analyser and 76% after transformation into dinitrophenylglycine) and ^{14}C -phenylacetic acid (as revealed by thin-layer chromatography on Silica-Gel plates using two solvents; Table 1). (2) ^{14}C -X and authentic phenylacetyl-glycine exhibited the same R_f values by thin-layer chromatography on Silica-Gel plates using three solvents (Table 1). (3) A mixture containing a few neq of ^{14}C -X and a 1,000-fold excess, on a molar basis, of non-radioactive phenylacetyl-glycine was submitted to three successive crystallisations from ethylacetate. Crystals thus obtained exhibited constant specific radioactivities. (4) The mass spectrum of ^{14}C -X was identical to that given by phenylacetyl-glycine (Fig. 1). (5) Both ^{14}C -X and phenylacetyl-glycine, esterified with CH_2N_2 , had the same retention time (6 min) by gas-liquid chromatography (at 200 °C, with H_2 and N_2 flow rates of 2 and 30 ml min^{-1} , respectively, and by using a capillary SP1000 column of length 20 m). Similarly, the ^{14}C -X compound obtained with the R39 enzyme was also characterised as ^{14}C -phenylacetyl-glycine by cocrystallisation and cochromatography with authentic phenylacetyl-glycine.

We conclude, therefore, that by interacting with benzylpenicillin, both R61 and R39 exocellular enzymes split the antibiotic molecule and that one of the fragments is phenylacetyl-glycine. Evidently the methylene carbon atom of the glycine residue must arise from C_6 of the penicillin nucleus and, presumably, the C_7 is retained as the glycine carboxyl group (Fig. 2). The fate of the thiazolidine nucleus of the penicillin molecule has not yet been determined. Chemically, benzylpenicillin methyl ester can be degraded to methyl D-5,5-dimethyl- Δ^2 -thiazolidine-4-carboxylate in trifluoroacetic acid. The N-phenylacetyl-glycyl fragment was isolated by conversion to its N-benzylamide⁸. A possible mechanism for the action of the *Streptomyces* enzymes would be that the initial event is a rupture of the β -lactam amide bond (Fig. 2; arrow a) with attachment of C_7 to some active group in the enzyme. By a process of β elimination, in which functional groups of the enzyme could participate,

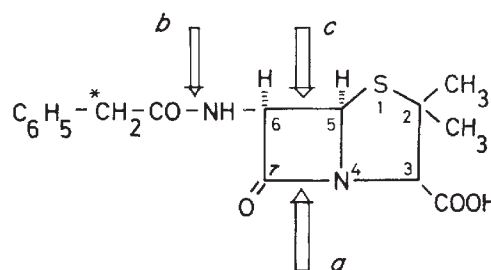


Fig. 2 ^{14}C -benzylpenicillin. a, Site of action of penicillinase (reaction product benzylpenicilloic acid); b, site of action of amidase (reaction product 6-aminopenicillanic acid); c and a, possible sites of action of the D-D-carboxypeptidase-transpeptidases of *Streptomyces* R61 and R39 (reaction product phenylacetyl-glycine + unknown). *, [^{14}C].

the link between C_5 and C_6 would become a double bond and further degradation would result in removal of the thiazolidine moiety (Fig. 2; arrow c). Release of the phenylacetyl-glycine would then occur. There may be other possible mechanisms and the problem is being studied at present.

Whatever the mechanism, however, our studies point to the great importance of the nature of the substituents on C_6 of the penicillin molecule. Substitution at C_6 drastically reduces the activity of the β -lactam antibiotics in all cases tested; 6-methoxy-penicillin derivatives, however, remain better inhibitors than the corresponding 6-methyl derivatives⁹. These studies may also be related with the observations of Schmid and Plapp¹⁰ that binding of penicillin to *Proteus mirabilis* was inhibited by phenylacetyl-glycine and that in the presence of this compound, formation of sphaeroplasts was prevented. Experiments have shown that the extracted and partially purified membrane-bound transpeptidases from *Streptomyces* R61 and *S. rimosus*⁵ also perform fragmentation of benzylpenicillin with formation of phenylacetyl-glycine (M. Leyh-Bouille, J. Dusart and J. M. Ghuysen, unpublished). Moreover, penicillin is known to be degraded into a biologically inactive compound by interacting with the membrane-bound D-D-carboxypeptidases of various *Bacillus* spp.¹¹. The fact that the membrane-bound targets of penicillin act as antibiotic-degrading compounds is important even if the overall process is slow. It leads to a better understanding of the phenomena of resistance which cannot be explained on the basis of production of either penicillinase or amidase (Fig. 2; arrows a and b, respectively). It may also be relevant to the necessity for the continuous supply of antibiotic during penicillin therapy.

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- ¹ Wise, E. B., Jr, and Park, J. T., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 75-81 (1965).
- ² Tipper, D. J., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 1133-1141 (1965).
- ³ Frère, J. M., Ghuyesen, J. M., Perkins, H. R., and Nieto, M., *Biochem. J.*, **135**, 463-468 (1973).
- ⁴ Frère, J. M., et al., *Biochem. J.*, **143**, 233-240 (1974).
- ⁵ Ghuyesen, J. M., et al., *Bull. Inst. Pasteur*, **73**, 101-140 (1975).
- ⁶ Frère, J. M., Ghuyesen, J. M., Reynolds, P. E., Moreno, R., and Perkins, H. R., *Biochem. J.*, **143**, 241-249 (1974).
- ⁷ Frère, J. M., Leyh-Bouille, M., Ghuyesen, J. M. and Perkins, H. R., *Eur. J. Biochem.*, **50**, 203-214 (1974).
- ⁸ Bell, M. R., Carlson, J. A., and Desterlin, R., *J. org. Chem.*, **37**, 2733-2735 (1972).
- ⁹ Ho, P. K., Townner, R. D., Indelicato, J. M., Spitzer, W. A., and Koppel, G. A., *J. Antibiot.*, Tokyo, **10**, 64-228 (1972).
- ¹⁰ Schmid, R., and Plapp, R., *Arch. Mikrobiol.*, **83**, 246-260 (1972).
- ¹¹ Blumberg, P. M., Yocum R. R., Willoughby, E., and Strominger, J. L., *J. molec. Biol.*, **249**, 6828-6835 (1974).

Scotopic and photopic dark adaptation of the b wave in isolated rat retina

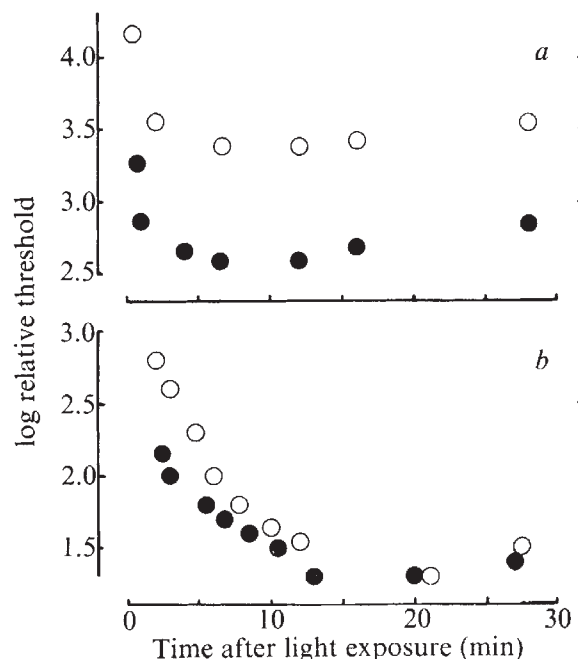
A SLOW form of rod dark adaptation lasting tens of minutes has been observed in isolated retinæ¹⁻³, including those of the rat^{6,7}, following exposures to intense lights which bleach significant proportions of the rod pigment. Yet in the isolated rat retina in similar circumstances it has been reported^{8,9} that the cells which produce the b wave of the electroretinogram adapt within minutes. Since this adaptation is finished before any of the slow thermal reactions of rhodopsin bleaching reach completion, a 'neural' basis for the phenomenon has been suggested. Experiments¹⁰ have, however, confirmed earlier work¹¹ showing that there is more than one pigment in the rat. This provides an alternative explanation for the fast adaptation of the rat b wave. We have been able to demonstrate that during the fast adaptation the dominant input to the cells producing the b wave comes from the non-rhodopsin pigment system. We have also found conditions in which the b wave shows the slower form of adaptation. Then, the dominant input to the b-wave cells is from a rhodopsin-based mechanism.

Figure 1a shows that following the extinction of an intense light which bleaches 70% of the rhodopsin in the retina, a very rapid adaptation can be seen in the b-wave threshold measurements, a similar phenomenon to that described previously^{6,8,9}. From about 6 min the threshold remains stable, except for a very slight decline in the preparation towards the end of 30 min. Frank and Dowling⁸ measured thresholds for periods of up to 1 h after a 50% bleach and also reported a stabilisation of the threshold. What is new in the present study is that we have demonstrated a permanent spectral shift towards the red in b-

wave sensitivity following the light exposure. The intensities of the red and blue lights have been equated for their effect on the b wave before the exposure, when, presumably, rhodopsin rods provided the dominant input to the b-wave cells. After the light exposure, the threshold intensities are no longer equal. This means that either bleaching thresholds for the rat b wave with lights of different wavelengths reaches the rhodopsin molecules, or the b-wave cells are controlled by a non-rhodopsin system. Our results are analogous to those of Green¹⁰ who studied increment thresholds for the rat b wave with lights of different wavelengths. He too observed a shift in spectral sensitivity towards the red for intense backgrounds, and since this occurred with flashed backgrounds which bleached insignificant amounts of pigment, he interpreted the shift in terms of a transition from scotopic to photopic control of the b wave. It is plausible to interpret our results in the same way. Our conclusion, then, is that the very fast adaptation which we and others have described, reflects processes in a photopic mechanism with a pigment more sensitive to red light than rhodopsin.

Although the b wave shows fast adaptation, the PIII response (the slow component following the b wave and having the opposite polarity) shows the slow adaptation which we have previously reported and which we believe may be linked with the formation of retinol and free opsin at the end of the rhodopsin bleaching sequence^{6,7}. PIII, as

Fig. 1 Adaptation of the b-wave threshold for blue (○) and red (●) stimuli following light exposures which removed 70% (a) and 18% (b) of the rhodopsin from the dark-adapted retinæ. Shimadzu interference filter, $\lambda_m = 482$ nm, used for blue light; Kodak, Wratten 25 filter for red light. b Waves in the range 5-30 μ V were elicited from the dark adapted retinæ with 50-ms blue and red flashes (frequency response of recording system 0.1-65 Hz). Estimates could thus be made of the calibrated neutral filters required with each type of flash to produce a criterion b wave. The retinæ were then exposed to 1 min (a) or 25 s (b) of unattenuated blue light. At various times following the extinction of the bleaching light, measurements were made of the neutral density values required to elicit criterion b waves. Each of these values was subtracted from the pre-bleach value to provide the data plotted above. Apparatus and preparation details have been given previously⁶. The solution used for perfusion had the following composition (mmol dm⁻³): NaCl, 119; KCl, 3.4; NaHCO₃, 21; NaH₂PO₄, 0.1; Na₂HPO₄, 0.8; MgSO₄, 2.4; CaCl₂, 2.0; and glucose, 28. It also contained 4% rabbit serum. It was bubbled with O₂-CO₂ (95:5) to give a pH between 7.6 and 7.9, and its temperature at the retina was 30.5 $\pm$ 0.5 °C.



measured here, is largely glial¹² and may have little to do with the signals sent by receptors to other neurones. The rat b wave on the other hand, though it too may have a glial origin¹³, is a good index of ganglion cell activity¹⁴. In our earlier study⁶, we therefore questioned how relevant the slow form of PIII adaptation was to understanding the adaptation processes of the rat visual system. Figure 1b shows, however, that the slow form of dark adaptation can also be seen in the rat b wave, provided the amount of rhodopsin bleached is relatively small. Although there is some shift in spectral sensitivity towards the red during the initial phase of adaptation, the final post-bleach spectral sensitivity is the same as that before the bleach. We conclude, therefore, that the slow form of adaptation is a property of rods containing rhodopsin. It seems to be very like the slow adaptation seen in the rods of other isolated retinæ¹⁻⁵. As Green¹⁰ found in his study on the rat, PIII is always dominated by the rhodopsin-based mechanism. The b wave (and presumably the higher order neurones) will also reflect rod phenomena including slow dark adaptation, provided small amounts of rhodopsin are bleached and the elevation of the threshold is relatively small. On the other hand, for larger bleaches, a photopic mechanism comes to control the b wave. For the living eye and for small pigment bleaches of the isolated retina, this may be transient, but for large bleaches of the isolated retina, the photopic input to the b-wave cells becomes a permanent feature.

These conclusions have implications in the way previous investigations of the rat b wave are interpreted. For example, it has been claimed that a general relationship of the form:

$$\log \text{threshold} \propto \text{fraction of pigment bleached}$$

underlies vertebrate dark adaptation. Work on the rat b wave represents one of the key pieces of evidence supporting this relationship (for a review, see ref. 15). The present study, however, makes it clear that many of the threshold measurements on the b wave in adaptation experiments reflect photopic activity and should not be correlated with the state of rhodopsin. Thus, the nature of the relationship between rhodopsin-based responses and the state of the pigment needs to be reconsidered.

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- ¹ Bender, S., and Baumann, C., *Pflügers Arch.*, **341**, 219–232 (1973).
- ² Frank, R. N., *Vision Res.*, **11**, 1113–1123 (1971).
- ³ Grabowski, S. R., and Pak, W. L., *J. Physiol., Lond.*, **247**, 363–392 (1975).
- ⁴ Hood, D. C., Hock, P. A., and Grover, B. G., *Vision Res.*, **13**, 1953–1963 (1973).
- ⁵ Witkovsky, P., Nelson, J., and Rippes, H., *J. gen. Physiol.*, **61**, 401–423 (1973).
- ⁶ Ernst, W., and Kemp, C. M., *Vision Res.*, **12**, 1937–1946 (1972).
- ⁷ Kemp, C. M., *Biochemistry and Physiology of Visual Pigments*, 307–312 (Springer, Berlin, 1973).
- ⁸ Frank, R. N., and Dowling, J. E., *Science*, **161**, 487–489 (1968).
- ⁹ Weinstein, G. W., Hobson, R. R., and Dowling, J. E., *Nature*, **215**, 134–138 (1967).
- ¹⁰ Green, D. G., *J. Physiol., Lond.*, **228**, 781–797 (1973).
- ¹¹ Dodt, E., and Echte, K., *J. Neurophysiol.*, **24**, 427–445 (1961).
- ¹² Ernst, W., and Arden, G. B., *Vision Res.*, **12**, 1759–1761 (1972).
- ¹³ Miller, R. F., and Dowling, J. E., *J. Neurophysiol.*, **33**, 323–341 (1970).
- ¹⁴ Weinstein, G. W., and Hobson, R. R., *Nature*, **227**, 957–959 (1970).
- ¹⁵ Rodieck, R. W., *The Vertebrate Retina*, 690–709 (Freeman, San Francisco, 1973).

Table 1 Fatty acid analyses of diets fed*

Fatty acid	Control	Diet SBOL	SSO
10:0+12:0+14:0	4.1	7.8	6.7
16:0+16:1	27.5	10.3	7.8
18:0	13.5	13.7	1.7
18:1	36.9	19.4	17.4
18:2ω6†	7.2	40.8	63.6
18:3ω3†	0.9	16.8	0.9
20:0+20:1	1.9	—	—
20:2ω6‡	0.1	—	—
20:3ω6‡	0.1	—	—
20:4ω6‡	0.5	—	—
20:5ω3‡	2.3	—	—
22:4ω6‡	0.1	—	—
22:5ω6‡	0.1	—	—
22:5ω3‡	0.2	—	—
22:6ω3‡	0.4	—	—
Total p-EFAs	8.1	57.6	64.5
Total d-EFAs	3.8	0.0	0.0

*As g per 100 g total fatty acids.

†p-EFA.

‡d-EFA.

—, Indicates compound not detected (limit of detection about 0.04 g per 100 g total fatty acids).

characterised by the position of the terminal double bonds relative to the methyl (omega) carbon atom. The ω6 series of EFAs all have double bonds in the ω6 and ω9 positions, the ω3 series in the ω3, ω6 and ω9 positions. The metabolic inter-relationships of the members of each series are shown in Fig. 1. The naturally occurring 18-carbon parent compounds for each series are linoleic acid (18:2ω6) and linolenic acid (18:3ω3) and EFA requirements are usually stated in terms of either or both of these parent EFAs (p-EFAs)^{1,3}.

Animal experiments, however, indicate that the higher homologues of the p-EFAs are the physiologically essential compounds¹. The dietary importance of the p-EFAs probably results solely from their role as precursors of these higher homologues, the derived EFAs (d-EFAs). Studies in the rat have shown that the desaturation steps involved in the production of d-EFAs do not always proceed at an optimal rate, being relatively easily inhibited by dietary or metabolic factors^{4,5}. We report here the results of dietary studies on the domestic cat which indicate that this species may lack the ability to further desaturate polyunsaturated fatty acids, and therefore exhibit a specific requirement for lipid of animal origin.

Cats (*Felis catus* L., domestic cross) were bred in this laboratory and when they weighed 0.8–1.7 kg were placed on purified experimental diets which avoided the use of animal lipids likely to be rich in d-EFAs. The two experimental diets used were based on casein (550 g kg⁻¹) and added oil (150 g kg⁻¹). This oil was safflower seed oil in the SSO diet; and a 5:1 (w/w) mixture of soybean oil and linseed oil in the SBOL diet. Both diets contained high levels of cholecalciferol (2.5 mg kg⁻¹ diet) but otherwise were designed to satisfy the known nutrient requirements of the cat^{6,7}. The control diet was a mixture of proprietary meat- and fish-based cat foods. It contained, on a dry weight basis, 450 g kg⁻¹ crude protein and 180 g kg⁻¹ lipid, and has proved satisfactory for maintaining our cat colony. The fatty acid composition of the diets is shown in Table 1. One male and one female were fed the SSO diet, two males and three females the SBOL diet and two males and two females the control diet for an average period of fifteen months (range 12 to 18 months).

The fatty acid composition of phosphoglycerides of plasma and red blood cells was examined by standard techniques⁸. The results obtained for plasma choline phosphoglycerides (CPGs) are shown in Table 2; ethanolamine phosphoglyceride and CPGs of red blood cells showed parallel changes. The tissue levels of d-EFAs were much reduced in experimental animals, only arachidonic acid (20:4ω6) and 11,14-eicosadienoic acid (20:2ω6) being present in detectable amounts. That these low levels occur in spite of the presence of very high amounts of

Inability of the cat to desaturate essential fatty acids

Most vertebrate species require some dietary source of essential fatty acids (EFAs)^{1,2}. A wide range of naturally occurring polyunsaturated fatty acids have been shown to exhibit EFA activity, but they can be classified into two homologous series

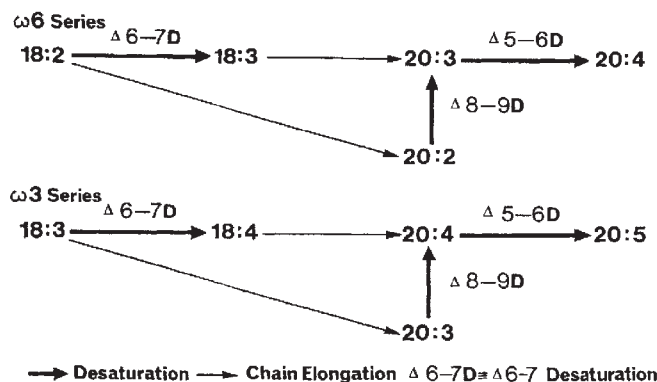


Fig. 1 Metabolism of the essential fatty acids (omitting the 22-carbon fatty acids).

18:2 $\omega 6$ in diet and tissue lipids, suggests that the experimental animals are unable to chain elongate or desaturate 18:2 $\omega 6$. Since 20:2 $\omega 6$ is not present in the diet, nor in the plasma CPG of normal cats, its presence in experimental animals demonstrates that chain elongation did occur. Studies in the rat have shown that the preferred pathway for the metabolism of 18:2 $\omega 6$ involves a $\Delta 6-7$ desaturation to 18:3 $\omega 6$ (Fig. 1). The chain elongation of 18:2 $\omega 6$ to 20:2 $\omega 6$ is relatively unimportant unless desaturation is impaired, therefore the detection of significant quantities of 20:2 $\omega 6$ and the virtual absence of desaturation products of 18:2 $\omega 6$ both imply that the $\Delta 6-7$ desaturase activity is lacking. Moreover, the absence of 8,11,14-eicosatrienoic acid (20:3 $\omega 6$) in spite of the high levels of 20:2 $\omega 6$ is indicative of the absence of $\Delta 8-9$ desaturase activity in the cat. Sprecher⁹ has recently reported that $\Delta 8-9$ desaturase activity is also absent in the rat.

Animals fed the SBOL diet seemed to have higher levels of 20:4 $\omega 6$ than the two animals receiving SSO diet, but in our routine gas liquid chromatography system 20:4 $\omega 6$ cochromatographs with 11,14,17-eicosatrienoic acid (20:3 $\omega 3$). By the use of a 50-m capillary column we were able to separate these components and show that the 20:4 $\omega 6$ peak contained about 40% 20:3 $\omega 3$. We failed to detect 20:3 $\omega 3$ in the 20:4 $\omega 6$ peak of animals fed SSO. We conclude that the plasma CPG levels of 20:4 $\omega 6$ were about the same in the two experimental groups and that the presence of 20:3 $\omega 3$ confirms the absence of $\Delta 6-7$ and $\Delta 8-9$ desaturase activity with $\omega 3$ fatty acids as a substrate.

The unknown fatty acid detected in plasma CPG of animals

fed all experimental diets has not yet been identified fully. We have established that it is a 20-carbon straight chain fatty acid and that it does not belong to the $\omega 3$, $\omega 6$ or $\omega 9$ series. Investigations into its structure are continuing.

Animals fed the experimental diets showed a varied clinical picture. All were listless, however, and had dry staring coats and severe dandruff. They grew poorly and seemed to be severely affected by infections and stress. Histological examination of their livers showed much fatty infiltration and parenchymal disorganisation. Both male and female animals became behaviourally infertile on the experimental diets, testes were underdeveloped and vaginal smearing failed to demonstrate the occurrence of oestrous cycles. We cannot explain these effects as a result of the level of dietary vitamin D, and, although limited by the lack of a suitable control group, there seems to be a similarity in this clinical picture with that caused in other species by feeding diets lacking in EFA activity^{1,10}. Few attempts have been made to feed cats defined diets for prolonged periods, but these have resulted in reduced growth and failure to breed¹¹. It may well be that deficiency of d-EFAs in purified diets caused these defects.

So far, one female SBOL animal has been returned to the control diet. This caused a rapid and sustained change in the fatty acid pattern of plasma CPGs (Table 3) and a marked improvement in clinical state. The animal rapidly gained weight (0.5 kg in 32 d), its coat became thicker and glossier and the dandruff disappeared. Its behaviour altered—the animal becoming more friendly and less restless. On day 32 of refeeding the animal came into oestrus and was successfully mated: after an uncomplicated pregnancy she gave birth to, and suckled, four normal young.

Table 3 Changes in essential fatty acids of plasma CPG in a SBOL cat refed control diet

Diet	SBOL	Control
Days on control diet	0	32
	Fatty acids g per 100 g total fatty acid	
18:2 $\omega 6$	48.3	34.6
18:3 $\omega 3$	2.6	0.9
Unknown	0.8	1.0
20:2 $\omega 6$	1.8	0.7
20:3 $\omega 6$	—	0.6
20:4 $\omega 6$ + 20:3 $\omega 3$	0.5	4.8
20:5 $\omega 3$	—	1.6
22:5 $\omega 3$	—	1.0
22:6 $\omega 3$	—	1.5
Total p-EFA	50.9	35.5
Total d-EFA	2.3	10.2

—, Indicates compound not detected (limit of detection about 0.04 g per 100 g total fatty acid).

Table 2 Fatty acid composition of plasma choline phosphoglycerides

	Control (n = 4)	SBOL fed (n = 5)	SSO 1	SSO 2
16:0 + 16:1	14.8 ± 0.05	10.2 ± 1.06	9.5	6.9
17:0	0.7 ± 0.06	0.2 ± 0.14	0.5	—
18:0	29.9 ± 0.64	26.3 ± 1.18	23.7	27.0
18:1	13.1 ± 0.85	9.5 ± 0.89	10.0	6.0
18:2 $\omega 6$	11.8 ± 0.86	45.6 ± 1.25	47.5	49.8
18:3 $\omega 3$	0.3 ± 0.11	2.6 ± 0.29	0.4	0.5
20:0 + 20:1	0.6 ± 0.12	0.7 ± 0.04	1.6	0.3
20:2 $\omega 6$	—	2.2 ± 0.24	4.9	6.2
Unknown	—	1.5 ± 0.25	1.6	3.0
21:0	0.1 ± 0.00	—	—	—
20:3 $\omega 6$	1.1 ± 0.15	—	—	—
20:4 $\omega 6$ + 20:3 $\omega 3$	14.3 ± 1.05	0.9 ± 0.14	0.3	0.3
20:5 $\omega 3$	8.0 ± 1.63	—	—	—
22:4 $\omega 6$	0.9 ± 0.34	—	—	—
22:5 $\omega 3$	0.8 ± 0.14	—	—	—
22:6 $\omega 6$	4.1 ± 0.39	—	—	—
Total p-EFA	12.1	48.2	47.9	50.3
Total d-EFA	29.2	3.1	5.2	6.5

Fatty acids as g per 100 g total fatty acids as mean (± s.e.m. where appropriate).

—, Indicates compound not detected (limit of detection about 0.04 g per 100 g fatty acids).

Our observations clearly indicate a lack of $\Delta 6-7$ and $\Delta 8-9$ desaturase activity in the cat. Preliminary results (J.R. and A.J.S., unpublished) indicate that the $\Delta 5-6$ desaturase activity is lacking so that conversion of 20:3 $\omega 6$ to 20:4 $\omega 6$ cannot occur.

We conclude that the EFA requirement of the cat can only be satisfied by a diet containing d-EFAs, regardless of the dietary levels of p-EFAs. This phenomenon has not previously been described in any animal species; however, a d-EFA requirement would explain an observation that animal lipid was a necessary dietary component for optimal health in mink¹². Classically, EFA deficiency is identified by an increase in tissue levels of 5,8,11-eicosatrienoic acid (20:3 $\omega 9$), a non-essential derivative of oleic acid synthesised by animals fed diets deficient in EFA. We did not observe 20:3 $\omega 9$ in our animals, a further indication of the absence of $\Delta 6-7$ and $\Delta 5-6$ desaturases. We postulate that a total EFA deficiency could be induced in the cat without significant levels of 20:3 $\omega 9$ being produced.

The absence of desaturase activity in the cat makes it a particularly good experimental model for research on prostaglandins

and essential fatty acid metabolism. The possibility that other species adapted to an omnivorous or carnivorous diet might show a d-EFA requirement also deserves investigation.

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¹ Alfin-Slater, R. B., and Aftergood, L., *Progr. biochem. Pharmac.*, **6**, 214–241 (1971).

² Stumpf, P. K., *A. Rev. Biochem.*, **38**, 159–212 (1969).

³ Davidson, S., Passmore, R., and Brock, J. F., *Human Nutrition and Dietetics*, fifth ed., 62–77 (Churchill Livingstone, Edinburgh and London, 1972).

⁴ Inkpen, C. A., Harris, R. A., and Quakenbush, F. W., *J. Lipid Res.*, **10**, 277–282 (1969).

⁵ Ninno, R. E., De Torrenzo, M. A. P., Castuma, J. C., and Brenner, R. R., *Biochim. biophys. Acta*, **360**, 124–133 (1974).

⁶ Gershoff, S. N., in *Nutrient Requirements of Laboratory Animals*, 1–10 (National Academy of Sciences, National Research Council, Washington, DC, 1962).

⁷ Scott, P. P., in *Canine and Feline Nutritional Requirements* (edit. by Graham-Jones, O.), 75–89 (Pergamon, Oxford and London, 1965).

⁸ Sinclair, A. J., and Crawford, M. A., *J. Neurochem.*, **19**, 1753–1758 (1972).

⁹ Sprecher, H., and Lee, C. J., *Biochim. biophys. Acta*, **388**, 113–125 (1975).

¹⁰ Holman, R. T., *Progr. Chem. Fats other Lipids*, **9**, 607–682 (1970).

¹¹ Greaves, J. P., thesis, Univ. London (1959).

¹² Leoschke, W. L., and Elvehjem, C. A., *J. Nutr.*, **69**, 147 (1959).

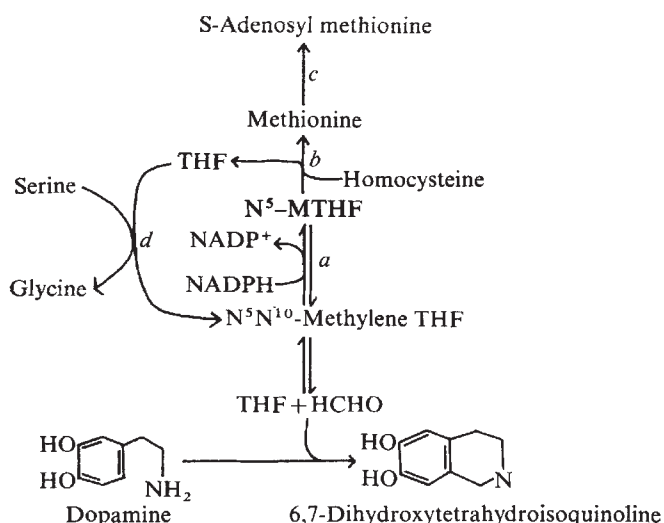


Fig. 1 Folate metabolism and tetrahydroisoquinoline synthesis. The enzymes involved are: *a*, methylenetetrahydrofolate reductase (EC 1.1.1.68); *b*, N⁵-MTHF homocysteine methyltransferase (EC 2.1.1.13); *c*, methionine adenosyltransferase (EC 2.5.1.6); *d*, serine hydroxymethyltransferase (EC 2.1.2.1). The dissociation of methylenetetrahydrofolate to tetrahydrofolate and formaldehyde and the reaction of formaldehyde with dopamine are presumed to be non-enzymic.

Folate-dependent 1-carbon transfer to biogenic amines mediated by methylenetetrahydrofolate reductase

It has been suggested that N-methylation of biogenic amines is involved in the development of certain schizophrenic disorders^{1,2}. Interest in this transmethylation hypothesis has been boosted by the reported identification of a mammalian N-methyltransferase ("dopamine methyltransferase"³) which uses as methyl donor N⁵-methyltetrahydrofolate (MTHF) rather than S-adenosyl methionine³⁻⁵. Further studies have demonstrated that the products of the incubation of catecholamines or indoleamines with MTHF and the transferase were not N-methylated amines but tetrahydroisoquinoline or tetrahydro- β -carboline alkaloids, respectively⁶⁻¹⁰. These compounds can be formed readily by the condensation of formaldehyde with the amines and it has therefore been postulated that the enzyme originally identified as a methyltransferase may in fact act to release formaldehyde from MTHF⁶⁻¹⁰. We provide evidence that the enzyme methylenetetrahydrofolate reductase (EC 1.1.1.68) is responsible for the reported production of formaldehyde from MTHF.

Methylenetetrahydrofolate reductase catalyses the NADPH-dependent reduction of N⁵,N¹⁰-methylenetetrahydrofolate to MTHF (see Fig. 1). In the reverse direction, in the presence of a suitable oxidant, MTHF is converted to methylenetetrahydrofolate which may dissociate to tetrahydrofolate and formaldehyde¹¹. The release of formaldehyde from MTHF may be used as an assay procedure, but the reductase may also be assayed spectrophotometrically in the direction of MTHF formation¹¹. In this work both assays have been used and dopamine methyltransferase was measured radiometrically as described by Laduron³.

High activities of both methylenetetrahydrofolate reductase¹¹ and dopamine methyltransferase³ are reported to occur in liver, and this tissue was used for most of the studies reported here. The reductase was purified to a specific activity of 0.1 U mg⁻¹ as described by Kutzbach and Stokstad¹¹ and the activities of the reductase and of dopamine methyltransferase copurified during this procedure. The product was further fractionated by gel filtration or isoelectric focusing.

Gel filtration was performed on a calibrated Sephadex G-200 column and the eluate was assayed for methylenetetrahydrofolate reductase and dopamine methyltransferase. Both activities coincided in a single symmetrical peak with an elution volume corresponding to an apparent molecular weight of 170,000–190,000 (Fig. 2a). Both methylenetetrahydrofolate reductase and dopamine methyltransferase were recovered in a single peak corresponding to an

Table 1 Effect of various additives on the activities of methylenetetrahydrofolate reductase and dopamine methyltransferase

Additive	Methylenetetrahydrofolate reductase activity (%)	Dopamine methyltransferase activity (%)
None	100	100
FAD (2×10^{-6} M)	410	320
DOPAC (1×10^{-3} M)	47	58
S-Adenosyl methionine (1.6×10^{-4} M)	< 10	< 10
S-Adenosyl methionine (1.6×10^{-4} M) + S-adenosyl homocysteine (1.6×10^{-4} M)	54	100

Methylenetetrahydrofolate reductase was purified from pig liver as described in the text and its activity was measured spectrophotometrically at 340 nm as described by Kutzbach and Stokstad¹¹. Dopamine methyltransferase was assayed radiometrically as described by Laduron³.

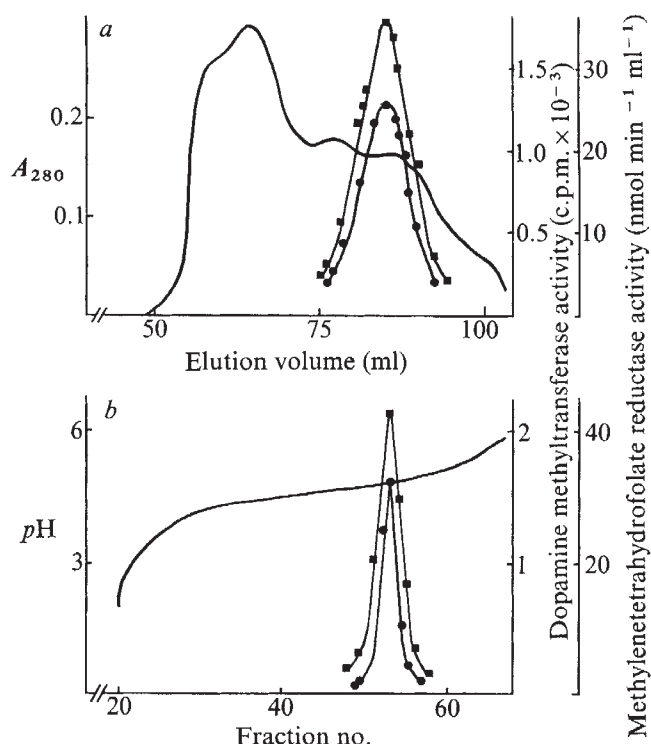


Fig. 2 *a*, Isolation of methylenetetrahydrofolate reductase by gel filtration on Sephadex G-200. 10 mg methylenetetrahydrofolate reductase (specific activity, 0.1 U mg⁻¹) purified from pig liver by the method of Kutzbach and Stokstad¹¹ was applied to a Sephadex G-200 column (1.5 × 95 cm) equilibrated in 10 mM potassium phosphate buffer, pH 7.5 containing 1 mM EDTA and 1 mM mercaptoethanol. Fractions were assayed for protein (*A*₂₈₀) and for methylenetetrahydrofolate reductase (●) and dopamine methyltransferase (■) activities as described in the text. *b*, Isoelectric focusing of methylenetetrahydrofolate reductase. 10 mg methylenetetrahydrofolate reductase was electrofocused at 4 °C in a 110 ml LKB column using ampholine, pH 3.5–5.0, in a 20–60% glycerol gradient. When electrofocusing was complete the column was drained and 2 ml fractions were collected. The pH value and the activities of methylenetetrahydrofolate reductase (●) and dopamine methyltransferase (■) of the fractions were measured. The recovery of enzyme activity was 50–60%.

isoelectric point of 4.8 (Fig. 2*b*). A similar result was obtained when dopamine methyltransferase, partially purified from bovine brain by the method of Laduron⁴, was assayed by isoelectric focusing.

Active fractions from the gel filtration column were pooled and concentrated and used to investigate the effects of several additives on enzyme activity. The assay procedure for methylenetetrahydrofolate reductase routinely includes the addition of 2 μM FAD which produces a fourfold stimulation of the rate observed in the absence of the flavin coenzyme¹¹ and, correspondingly, the activity of dopamine methyltransferase was shown to be significantly increased in the presence of FAD (Table 1). 3,4-Dihydroxyphenylacetic acid (DOPAC) has been reported to inhibit the transferase¹². We observed that 10⁻² M DOPAC inhibited both the transferase and methylenetetrahydrofolate reductase activities to similar extents (Table 1).

Methylenetetrahydrofolate reductase is the initial enzyme in the pathway leading to the synthesis of S-adenosyl methionine (see Fig. 1). The reductase is allosterically inhibited by S-adenosyl methionine and this inhibition is relieved by S-adenosyl homocysteine¹¹. As shown in Table 1, 1.6 × 10⁻⁴ M S-adenosyl methionine inhibited both the reductase and the transferase to similar extents and this inhibition was indeed reversed by S-adenosyl homocysteine.

The evidence presented here therefore supports the hypothesis that methylenetetrahydrofolate reductase and dopamine methyltransferase represent two activities exhibited by a single enzyme species. It is noteworthy that the regional distribution of these two activities in brain have previously been shown to be similar^{13,14}. The mechanism of 1-carbon transfer to biogenic amines by the reductase *in vitro* would seem to involve oxidation of MTHF to methylenetetrahydrofolate and subsequent non-enzymic dissociation of formaldehyde from the folate derivative (Fig. 1). It is, at present, uncertain whether mediation in the synthesis of tetrahydroisoquinoline and tetrahydro-β-carboline alkaloids represents a physiological activity of this enzyme. Both the equilibrium of the reaction¹¹ and the NADPH-NADP⁺ ratio in the cytosol¹⁵ would favour MTHF synthesis rather than the reverse reaction involving formaldehyde release. Furthermore, free formaldehyde may also bind to tissue protein¹⁶ or be oxidised by formaldehyde dehydrogenase¹⁷. The feedback inhibition of methylenetetrahydrofolate reductase by S-adenosyl methionine also supports the concept that the physiological function of this enzyme is in the ultimate synthesis of S-adenosyl methionine rather than the metabolism of biogenic amines. Since the formation of pharmacologically active alkaloids¹⁸ from MTHF and amines may be detected *in vitro*, however, it would seem to be important to investigate their possible formation *in vivo*.

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- Matthysse, S., Smith, E. L., Puck, T. T., and Edelman, G. M., *Neurosci. Res. Progr. Bull.*, **10**, 446–445 (1972).
- Snyder, S. H., and Banerjee, S. P., in *Frontiers in Catecholamine Research* (edit. by Usdin, E., and Snyder, S. H.), 1133–1138 (Pergamon, Oxford, 1973).
- Laduron, P., *Nature new Biol.*, **238**, 212–213 (1972).
- Laduron, P. M., Gommeren, W. R., and Leysen, J. E., *Biochem. Pharmacol.*, **23**, 1599–1608 (1974).
- Banerjee, S. P., and Snyder, S. H., *Science*, **182**, 74–75 (1973).
- Leysen, J., and Laduron, P., *FEBS Lett.*, **47**, 299–303 (1974).
- Meller, E., Rosengarten, H., Friedhoff, A. J., Stebbins, R. D., and Silber, R., *Science*, **187**, 171–173 (1975).
- Lin, R. L., and Narasimhachari, N., *Res. Commun. chem. Path. Pharmacol.*, **8**, 535–541 (1974).
- Mandel, L. R., Rosegay, A., Walker, R. W., Vanden Heuvel, W. J. A., and Rokach, J., *Science*, **186**, 741–743 (1974).
- Wyatt, R. J., Erdelyi, E., Doamaral, J. R., Elliott, G. R., Renson, J., and Barchas, J. D., *Science*, **187**, 853–855 (1975).
- Kutzbach, C., and Stokstad, E. L. R., *Biochim. Biophys. Acta*, **250**, 459–477 (1971).
- Waldmeier, P. C., and Maitre, L., *Experientia*, **30**, 456–458 (1974).
- Burton, E. G., and Sallach, H. J., *Archs Biochem. Biophys.*, **166**, 483–494 (1975).
- Mandell, A. J., Knapp, S., and Hsu, L. L., *Life Sci.*, **14**, 1–17 (1974).
- Jacobson, K. B., and Kaplan, N. O., *J. biol. Chem.*, **226**, 603–613 (1957).
- Ungar, F., Tabakoff, B., and Alivisatos, S. G. A., *Biochem. Pharmacol.*, **22**, 1905–1913 (1973).
- Uotila, L., and Koivusalo, M., *J. biol. Chem.*, **249**, 7653–7663 (1974).
- Davis, V. E., *Ann. N. Y. Acad. Sci.*, **215**, 111–115 (1973).

Errata

In the Matters Arising contribution "Development of visual acuity and the sensitive period" by J. T. Flynn, D. I. Hamasaki, T. E. Flynn and M. Barricks (*Nature*, **257**, 337; 1975) the author of the original article quoted in ref. 1 should be D. N. Freeman and not R. D. Freeman.

In the article "Superior growth of the right gonad in human foetuses" by U. Mittwoch and D. Kirk (*Nature*, **257**, 791; 1975) the second sentence of the legend to Fig. 1 should begin: Bars represent the means of the . . . and not as printed.

matters arising

Lunar magnetism

IN a recent series of papers, Runcorn^{1,2} has maintained that the observation of a very small lunar surface dipole field ($\lesssim 0.05\gamma$) implies that there used to be a fairly strong interior lunar dipole moment ($\gtrsim 3.2 \times 10^9$ yr ago). He contends that if such a field had disappeared in the past 3.2×10^9 yr, the exterior field of the Moon would now be zero. This, he argues, is a direct result from potential theory.

I show, for a very simple model of the Moon, that if a primordial core magnetic field existed, it would give rise to a present day non-zero dipole external field.

Consider a uniformly magnetised core of radius a , embedded in a permeable mantle with outer radius b . The core magnetisation is $M_0 = M_0 \hat{e}_3$ and the scalar potential of the magnetic field, H , satisfies $H = -\nabla\phi(x)$, with $B \sim \mu H$ in the mantle. The boundary value problem is easily solved with the result.

$$\Phi_C(x) = ar \cos\theta \quad (1)$$

$$\Phi_M(x) = (\beta r + \gamma/r^2) \cos\theta \quad (2)$$

$$\Phi_V(x) = \delta/r^2 \quad (3)$$

where C, M, and V refer to core, mantle, and vacuum, respectively; and

$$\begin{aligned} \alpha &= \beta + \gamma/a^3 \\ \beta &= -2(1-\mu)A \\ \gamma &= b^3(\mu+2)A \\ \delta &= 3\mu b^3A \\ A &= 4\pi M_0 a^3/D \\ D &= (2\mu+1)(\mu+2)b^3 - 2a^3(1-\mu)^2 \end{aligned} \quad (4)$$

Now imagine that the magnetising currents in the core die out.

The magnetisation of the mantle in the absence of a core field is then

$$\begin{aligned} M(x) &= \frac{(\mu-1)}{4\pi} \left[-\beta + \frac{2\gamma}{r^3} \right] \cos\theta \hat{e}_r + \\ &+ \left[\beta + \frac{\gamma}{r^3} \right] \sin\theta \hat{e}_\theta \end{aligned} \quad (5)$$

The scalar potential, $\Psi(x)$, of the resulting field is

$$\begin{aligned} \Psi_C(x) &= -\frac{2}{3} \frac{(\mu-1)}{a^3} (1-a^3/b^3) \times \\ &\times \gamma r \cos\theta \end{aligned} \quad (6)$$

$$\begin{aligned} \Psi_M(x) &= \frac{(\mu-1)}{3} [\beta(a^3-r^3) - \\ &- 2\gamma(1-r^3/b^3)] \frac{\cos\theta}{r^2} \end{aligned} \quad (7)$$

$$\Psi_V(x) = \frac{(\mu-1)}{3} (b^3-a^3)\beta \frac{\cos\theta}{r^2} \quad (8)$$

Equation (8) leads to a non-zero external dipole field. Runcorn's conclusion^{1,2}, that the external field is zero, is based on his assertion that the potential of the magnetising field has the form of equation (2), but with $\beta = 0$. Clearly, if $\beta = 0$ in equation (2), then $\Psi_V(x) = 0$. It must be emphasised that, using an

of the magnetostatic field equations. The solution of the problem with zero core field can be obtained from the solution with non-zero field, equations (1)–(4), by adding to the fields derived from equations (1)–(4), the field of a uniformly magnetised sphere of radius a , with magnetisation $M' = -M_0 \hat{e}_3$. This is indicated schematically in Fig. 1. The resulting external field will clearly be a dipole of reduced strength. The fields resulting from such a superposition are in fact identical to those resulting from equations (7) and (8).

Stephenson *et al.*³ note that Runcorn's result is strictly true only if the magnetic susceptibility of the mantle is very small, $\sim 10^{-4}$. Although it is clear that the exterior field is of higher order in $(1-\mu)$ than the fields in the other two regions, one must be cautious about arguing that it is therefore negligible. The permeability has been treated as though it were paramagnetic in this simple derivation, but it must, of necessity, be ferromagnetic. To my knowledge the ferromagnetic permeability of the Moon is not known. Dyal *et al.*⁴ have, however, found a paramagnetic permeability $\mu \sim 1.01$, that already is larger than the value of $1 + 10^{-4}$ used by Stephenson *et al.*³. This larger value for μ implies the existence of ferromagnetic material⁴, the properties of which are undetermined.

I acknowledge discussions with Dr N. F. Ness and J. D. Scudder.

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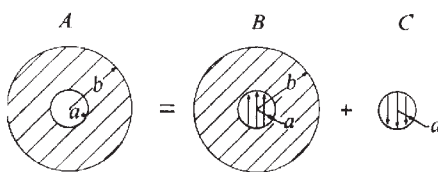


Fig. 1 The superposition of the magnetic fields. The field in A where there is no core magnetisation can be derived by subtracting the field of a uniformly magnetised sphere, radius a , (C) from that of the large uniformly magnetised sphere (B). See the equations for details.

internal magnetising field, the external field is in general non-zero after the core field has decayed to zero. The solution given by equations (6)–(8) is, in fact, a linear combination of the 'interior' and 'exterior' solutions discussed by Runcorn².

The conclusion that the external field is not zero also follows from the linearity

RUNCORN REPLIES—The theorem^{1,2} which I proved is exactly correct as I stated it: that is, if a spherical shell of any thickness acquires permanent magnetisation, the intensity of which is proportional and parallel to a magnetising field of internal origin, which later disappears, its external field is zero. In the simpler proof¹, the magnetising field was assumed to be that

¹ Runcorn, S. K., *Nature*, 253, 701 (1975).

² Runcorn, S. K., *Phys. Earth planet. Interiors* (in the press).

³ Stephenson, A., Runcorn, S. K., and Collinson, D. W., *Proc. Sixth Lunar Sci. Conf.* (Pergamon, New York, in the press).

⁴ Dyal, P., Parkin, C. W., and Daily, W. D., *Proc. Sixth Lunar Sci. Conf.* (Pergamon, New York, in the press).

of a centred dipole and I used the theorem to show that the new determination³ of the present lunar dipole magnetic field as $< 1.3 \times 10^{18}$ gauss cm³, or $< 0.05 \gamma$ at the equator (essentially negligible), is very powerful evidence for the existence of an ancient lunar magnetic field of internal origin, which had been postulated to explain the natural remanent magnetisation discovered in the lavas returned by the Apollo missions⁴, and effectively excludes an external agency unless it resulted in random magnetisation.

It was shown² that if the maximum magnetisation of the shell at its exterior is I , then, for the dipole case, there is within its inner boundary, a uniform field of intensity $4\pi I/3$ times the ratio of the volume of the shell to that of the unmagnetised inner sphere. If the depth of the Curie point isotherm is taken as 50 km, this ratio is 0.1, and if $I = 10^{-4}$ e.m.u. g⁻¹ the uniform field is 10γ .

I was aware that such a field, derived from the outer part of the magnetised shell, not only induces magnetisation in its interior, the point of Goldstein's communication, but would also have modified the magnetising field in which the rocks, cooling through the Curie point, acquired thermoremanent magnetisation. The resultant component of uniform magnetisation of the shell, increasing inwards, will give rise to an external dipole field, as will the departure of the Moon from sphericity, but I considered these effects and found them negligible². Goldstein incorrectly quotes us in the last paragraph: we⁶ stated that, for the theorem to be applicable, c , the magnetisation acquired in unit field, must be very small, of the order of 10^{-4} , not the magnetic susceptibility ($\mu - 1$) which even if it is 10^{-2} , as one analysis gives⁷, produces a dipole field of much less than the observable limit. As the equatorial surface field of a shell, of uniform magnetisation I' , is $4\pi I'/3$ multiplied by the ratio of the volumes of the shell to the sphere, this external field is about 0.01γ for the Moon.

In devising a mathematical model to describe and understand the significance of experimental data, the scientist simplifies the physical situation by leaving out of consideration factors which are unimportant. Lytleton⁸ has lately reminded geophysicists of Eddington's description of the process. It is entirely legitimate for the mathematician to point out that the most general expression has not been used, but he must show quantitatively that with this added complexity, observations can be better fitted or the inferences to be drawn are thereby qualitatively changed; Goldstein does neither. But perhaps when the accuracy and reliability of magnetometers carried in spacecraft is improved by orders of magnitude, the further refinement of the theorem will be necessary. Goldstein may be drawing an analogy with the Earth—often misleading for lunar scientists—

where the induced magnetisation of geological formations is usually of the same order as the permanent magnetisation.

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- ¹ Runcorn, S. K., *Nature*, **253**, 701–702 (1975).
- ² Runcorn, S. K., *Phys. Earth planet. Interiors*, **10**, 55–62 (1975).
- ³ Russell, C. T., Coleman, P. J., and Schubert, G., *Science*, **186**, 825–826 (1974).
- ⁴ Runcorn, S. K., et al., *Geochim. cosmochim. Acta*, suppl. 1, **3**, 2369–2387 (1970); Runcorn, S. K., et al., *Proc. R. Soc.*, **A325**, 157–174 (1971).
- ⁵ Goldstein, M. L., *Nature*, **258**, 175 (1975).
- ⁶ Stephenson, A., Runcorn, S. K., and Collinson, D. W., *Geochim. cosmochim. Acta*, suppl. 6, **3**, (in the press).
- ⁷ Dyal, P., Parkin, C. W., and Daily, W. D., *Geochim. cosmochim. Acta*, suppl. 6, **3** (in the press).
- ⁸ Lytleton, R. A., *Adv. Astr. Astrophys.*, **7**, 83 (1970).

Effects of gallic acid on nitrosamine formation

OBSERVATIONS¹ on the catalytic effect of the naturally occurring polyphenol, chlorogenic acid, on the nitrosation of piperidine, may be supplemented by similar observations we have made during an investigation into the effect of gallic acid—an essential constituent of the gallotannins—on nitrosation of diethylamine. The possibility that such compounds could exert an influence on the *in vivo* formation of carcinogenic nitrosamines deserves careful attention.

Though early experiments² established the existence of an impeding effect by tannins on the formation of nitrosamines, more recent work shows that the amount of nitrosamine formed is dependent both on the pH and the concentration of the gallic acid.

We reacted concentrations of nitrite (0.075 M) and diethylamine (0.5 M) in buffered aqueous solutions, in the presence of different concentrations of gallic acid. Reaction was allowed to proceed for 30 min after which the solution was made alkaline to stop further reaction, and extracted with methylene chloride. The concentration of nitrosamine was determined using both ultraviolet spectrophotometry and gas chromatography. The concentrations of both the nitrite and the amine were chosen to give a convenient yield of nitrosodiethylamine for measurement of both systems. The relatively high concentration of amine allowed the use of a lower concentration of nitrite so that the competitive reaction of nitrite with gallic acid was not masked.

The results show (Fig. 1) that although gallic acid does exert a catalytic effect, it is extremely dependent on pH and is restricted to a narrow range in the region of pH 4. In addition, the balance between the

retarding and catalytic effect is strongly dependent on the concentration of gallic acid, as illustrated by the fact that an increase in the formation of nitrosamine related almost linearly with a decrease in the concentration of gallic acid. Similar effects were also found when solutions of tannic acid were used in place of gallic acid.

Though this tends to support the views of Challis and Bartlett¹, the idea that a catalytic effect by natural phenols can lead to the *in vivo* formation of nitrosamines, should be considered with caution. Nitrosamine formation could be dependent on the

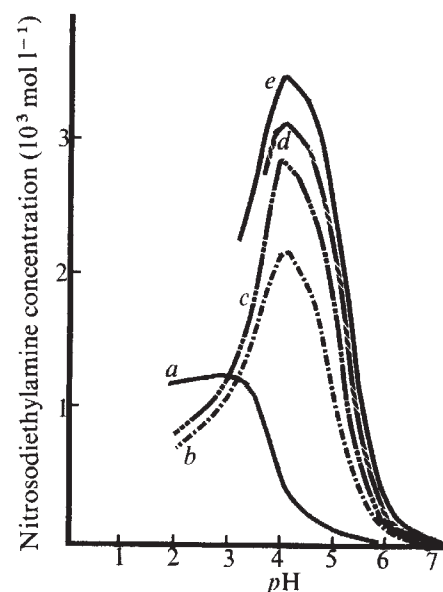


Fig. 1 Diagram demonstrating the increasing catalytic effect of gallic acid on nitrosodiethylamine formation with decreasing concentrations of gallic acid. Gallic acid concentration: a, 0; b, 0.0625 M; c, 0.0375 M; d, 0.025 M; e, 0.0125 M.

variable pH conditions in the digestive system and on eating and drinking habits which could regulate the simultaneous concentrations of nitrite, tannins and nitrosatable amines that may have been ingested or formed in the system.

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¹ Challis, B., and Bartlett, C. D., *Nature*, **254**, 532–533 (1975).

² Bogovski, P., Castegnaro, M., Pignatelli, B., and Walker, E. A., *N-Nitroso Compounds Analysis and Formation*, No. 3, 127 (IARC Scientific Publications, 1972).

reviews

THE capacity of those individuals who survive infectious diseases to become immune to further attack was recognised early in history, and for several hundred years attempts have been made to exploit this phenomenon in medicine. It is nearly one hundred years since Behring showed that the substances which are responsible are present in the blood of the immune animal and named them antibodies, but it is less than twenty years since advances in knowledge of protein chemistry made it possible to isolate and study the structure of these remarkable molecules. Their fascination lies in the ability of almost identical molecules to combine specifically with an apparently endless range of different substances and the recognition that this phenomenon is essential for the survival of all species of vertebrates. The growing realisation that a related phenomenon may be the basis of cellular recognition and therefore be an important feature of differentiation has put the antibody molecule in a key position in biological research. Here for the first time, the variable region of antibody molecules offers a chemical basis for one aspect of the infinite variety of living things. The genetic origin of this very wide range of protein structures found in all individuals remains a mystery, but one likely to be solved soon.

Even so, it is surprising to find in this book just how much information has been acquired in so short a time. The emphasis throughout the book is on the structure of the immunoglobulins, the proteins which carry the antibody activity. The chemical structures which

Antibody molecule

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The Antibody Molecule. (Immunology: An International Series of Monographs and Treatises.) By Alfred Nisonoff, John E. Hopper and Susan B. Spring. Pp. xiv+542. (Academic: New York, San Francisco and London, August 1975.) \$34.50; £16.55.

define the division of the immunoglobulins into class and subclass, type and subtype, the different polymorphic forms, and the amino acid sequence changes found in the variable regions are all well described. Most of this work is soundly based, and although much remains to be investigated sufficient detailed study has been carried out on the immunoglobulins of several species to provide a body of knowledge which is now the basis for present investigations on the genetics, the control of synthesis and cellular origins of immunoglobulin and on many other aspects of immunity.

The authors have written an advanced monograph in which a mass of complex data has been presented in a well ordered and comprehensible manner. Sufficient detail is given to

make this book an excellent source of reference for those working in the field, but it never obscures the main themes. It should be of equal value for the many biologists with a peripheral interest in the subject. The book has been published quickly: most of the X-ray crystallographic studies of the past year or two are described and a most informative coloured plate has been included of the model of the *Fab* fragment of a human myeloma protein. Enough experimental detail will be found to support the results described and to explain, for example, the discovery of the still somewhat obscure idiotypic specificities of antibodies.

Most of the work described is of immediate interest, but some, such as the early attempts to define the relative contribution of the heavy and light chain of antibodies to the structure of the combining site, have a dated flavour and already seem to have been superseded by subsequent knowledge of the chemistry and crystallographic studies of the variable regions. Succinct accounts are included of the brief histories of most of the topics discussed and are incomplete only in the over-modest absence of references to the senior author. Careful reading of the extensive bibliographies at the end of each chapter is necessary to appreciate how large a contribution to our knowledge of antibody structure has been made by Dr Nisonoff.

This book gives a clear account of our factual knowledge of the structure of antibodies and of its biological significance. It is likely to remain of enduring value for some years to come.

WE have been here many times before—from early man and increasing plunder of wildlife to population explosion and pollution—but this is a very readable addition to the considerable clutch of similar books.

The detailed effects of man's impact on wildlife (and vice versa) are discussed in convenient form. There are chapters on plants and topography, the sea, marine mammals, birds, insects, and other invertebrates, followed by one on "Conservation Profit and Loss" and a rather curious bibliography for further reading.

The authoritative and lively style is to be welcomed. Not so, instances

Man and wildlife

Man and Wildlife. (Biology and Environment.) By L. Harrison Matthews. Pp. 129+16 plates. (Croom Helm: London, September 1975.) £4.95.

of what seems to be prejudice leading to dubious generalisation and emotive references out of tune with the character of the book. As an example, "superficial" wildlife films on television as well as bird watchers with their "little" lists are castigated with-

out acknowledgement that herein may lie the genesis of funds for holding open the conservation options for future generations—to purchase Wash and Lake Nakuru mud, and to pay for 'Operation Tiger' and education all over the world.

The Publishers' note on the wrapper expressing some hope for wildlife's future is clearly not endorsed by the author in his concluding chapter which omits adequate analysis of the conservation effort. Yet if we are to accept that all hope is lost at this stage, much of the *raison d'être* of civilised man is lost also.

Derek Barber

Cognitive aspects of reading

The Psychology of Reading. By Eleanor J. Gibson and Harry Levin. Pp. xii+630. (MIT: Reading, Massachusetts and London, 1975.) \$18.95.

THE republication of Huey's *Psychology and Pedagogy of Reading* after sixty years of general reticence on the topic has been accompanied by a rush of modern treatments. This rebirth of interest in reading reflects not only the cunning propensity of psychology for crop rotation but also an increasing interest of cognitive psychologists in skilled and intelligent performance. At the same time social concern about literacy, especially in the US, has enabled the psychologist, like a modern Danaë, to be possessed by Zeus in a shower of research funds. This volume by Gibson and Levin presents the most comprehensive engagement to date of cognitive psychology with the problem of reading.

The book consists of three sections, the first of which reviews the basic psychological and linguistic principles which the authors believe relevant. These include perceptual learning, work on general, cognitive and especially language development, basic linguistics and some specialised material on writing systems, and finally, a discussion of the considerable literature on the visual perception of words.

It is this extensive review section which sets the book apart and, in a sense, exhibits its academic credentials. The result is not entirely satisfactory. One problem is that the survey has something of the texture of 'annual review' articles, dense and impressively referenced but somehow lacking in coherence. The mass of data is insufficiently organised to be readily digested by the non-psychologist or to provide new insight for the specialist. Significantly, the introductory chapters on linguistics and writing systems are much more readable than those reviewing areas of experimental psychology. There are some obscurities, such as the curious description of Spoehr and Smith's research on pages 91-92 and the seemingly contradictory claims about the effect of orthographic regularity on letter search. But on the whole the text is dry and lucid.

The treatment of underlying concepts is lengthy yet some judgements have been made about exclusion of material. Notable absences include vision research such as metacontrast, much of the post-Sperling work on letter array processing and the important work of Meyer on lexical deci-

sions. There is also little explicit treatment of general problems of pattern recognition.

The core of the book is the section on the reading process itself. This is rather more readable, not least because the view of basic questions is less obscured by an untidy forest of experimental data. This section should also prove fruitful to the non-specialist. It concludes with a review of models of skilled reading, which brings the discussion back to the preoccupations of experimental psychology. Here, in an attempt to invent some substantive issues the authors create a curious opposition of 'information processing' and 'analysis-by-synthesis' models. This chapter also contains one of the occasional lapses into slightly partial reviewing, where (page 467) the claims of Gibson and her colleagues about an absence of acoustic or semantic effects on visual search are presented without

mention of conflicting evidence.

The final section consists of short capsules for such questions as dyslexia and speed reading. Of these the freshest and most absorbing is on cross-language differences in reading achievement. Here is set out the baffling lack of well established relationship between reading development and the vastly different forms of orthography. This is almost enough to terminate the cognitive psychologist's flirtation with the real world. One echoes the words of a recent article title: "Do not adjust your mind, there is a fault in reality."

Huey's classic book is a more provocative essay on reading and the recent experimental literature is more penetratingly treated elsewhere, but Gibson and Levin have produced a scholarly and comprehensive textbook. Like boxed sets of symphonies there is a simple virtue in completeness.

Leslie Henderson

Life gaining ground

Surtsey: Evolution of Life on a Volcanic Island. By Sturla Fridriksson. Pp. 198. (Butterworth: London, 1975.) £5.00.

ON November 14, 1963, a submarine volcanic eruption began in the ocean south of the Westman Islands off the coast of Iceland, which eventually resulted in the production of an island with an area of 2.5 square kilometres. The island was aptly named Surtsey, after the mythical Norse fire-giant, Surtur the Black. The geological significance of this rare event was immediately apparent, but the biological opportunities presented by the occurrence are of no lesser importance. These were fortunately recognised by men such as Sturla Fridriksson, who visited the island within six months of its formation and began the formidable task of documenting the establishment of living organisms.

The succession of life on totally bare areas of land surface has not been studied intensively, mainly because of the lack of opportunity, especially in isolated situations where invasion demands very effective dispersal on the part of the organism concerned. Students must surely be weary of the much repeated but rather fragmentary information available from Krakatoa following its eruption in 1883. But in this new book, Fridriksson has summarised the development of living communities of organisms and simple ecosystems in the 10 years since the formation of Surtsey.

The study is extensive. It documents the establishment of microbial life within months of the initial eruption,

and the arrival of the first invertebrate terrestrial animals, birds and viable seeds. Complete lists of plant invaders (including bryophytes) are given, with details of when they arrived. Bird life is also covered in detail, the book largely being concerned with visiting species, but also recording with some pride the establishment of breeding pairs of fulmar and black guillemot. Arthropods are dealt with in less detail; 158 species have now been recorded, but many of these could have failed to establish themselves as permanent inhabitants.

The chapter on "Ways of Dispersal" is particularly interesting in that it deals with mechanisms by which dispersal (mainly botanical) has been effected. Some species, such as *Senecio vulgaris* and *Eriophorum scheuchzeri* have been observed during invasion by air-borne seeds. A surprising list is given of seeds found attached to the egg cases of skate and washed up on the island; many grasses arrived in that manner. Some seed eating birds (snow buntings) were found to have viable seeds in their gizzards, which could potentially provide an invasion mechanism.

Finally, the developing biota of Surtsey is compared with neighbouring islands and the mainland of Iceland, and naturally these seem to provide the major source areas for invaders.

The book is well illustrated with some spectacular colour photographs as well as a large number of line drawings and maps. It is written in a lively, informal manner, which effectively conveys Fridriksson's vigour and his enthusiastic concern to observe the ecological development of a truly pristine habitat.

P. D. Moore

Liquid column chromatography

Liquid Column Chromatography: A Survey of Modern Techniques and Applications. (Journal of Chromatography Library, Volume 3.) Edited by Zdenek Deyl, Karel Macek and Jaroslav Janak. Pp. xxi+1,175. (Elsevier Scientific: Amsterdam, London and New York, 1975.) Dfl. 290; \$120.95.

THIS collaborative 1,120-page volume, almost entirely the work of a group of Czech authors, is undoubtedly the most comprehensive account of the subject currently available. It is divided into four main sections: theoretical aspects of liquid chromatography, techniques of liquid chromatography, practice of liquid chromatography, and applications. The first three sections are general and make up about one-third (413 pages) of the book, whereas the application of these methods to specialised classes of compounds make up the remaining two-thirds.

The general theoretical section is in some respects the least successful part of the book, as in my opinion too much attention is paid to mathematical rigour and insufficient space (only some 10 pages) to the factors which influence zone resolution in practical chromatography. The short section on the thermodynamics of partition and adsorption processes by Novak, however, is excellent. It is rather disappointing to find the old but fallacious concept of a direct relation between solute molecular weight and retention volume adopted in all the sections on gel permeation (or molecular-sieve) chromatography.

The second and third sections on the techniques and practice of liquid column chromatography are excellent. The editors explain that they have decided, wisely in my view, to give equal prominence to the "classical" methods of liquid chromatography and to the newer techniques of high efficiency, high pressure chromatography, especially as the former still constitute 90% of published methods. The techniques section is essentially confined to instrumentation and materials (mobile and stationary phases) for all the fundamental subgroups of chromatography, adsorption, partition, ion-exchange and the exciting new development of affinity chromatography. The chapters (8 and 11) on instrumentation and operation of high efficiency, high pressure systems compare favourably with some of the specialised works on the subject. Chapter 9 on stationary phases and chapter 10 on mobile phases are particularly valuable, and are admirably

complemented by the corresponding chapters 13 and 14 in the practice section. These sections include extensive tables of the properties of the relevant stationary and support phases, as well as those of mobile phase solvents. The criteria for choice of phase, and packing, conditioning and operation of column receive special attention. The sections throughout the book contributed by Dr O. Mikes on ion-exchange chromatography and by Mrs J. Turkova on affinity chromatography must be specially commended.

The special section of the book gives detailed recommendations for the chromatographic separation of a variety of chemical compounds ranging through hydrocarbons, alcohols through carbohydrates, lipids, amino acids peptides and proteins, nucleic acids and their constituents to some specialised classes of organic compounds such as vitamins, antibodies and pesticides, to cells and subcellular particles, and finally to inorganic compounds.

It is always difficult to assess the value of such large specialised sections

in any book, as only a small portion of the material presented will be of any value or even interest to any particular reader, and also such material is more likely to become outdated than general sections. I believe that the best solution is a series of well-documented and indexed tables which will guide the interested reader into the literature of his own subject, without increasing the bulk (and cost) of the book with specialised material of no interest to him.

Among the best of these special chapters are 32 on amino acids by Zmrhal, Heathcote and Washington, 34 on peptides by Khluch, 35 and 36 on proteins by Prusik and Mikes, and 37 and 38 on nucleic acids and their constituents by Zadrazil. These are virtually monographs on their subjects.

In summary, this is by far the best book on liquid column chromatography to appear to date. It is excellently produced, but it is unfortunate that its price will render it inaccessible to many departmental libraries.

C. J. O. R. Morris

Dynamics of galaxies

The Formation and Dynamics of Galaxies—Proceedings of IAU Symposium No 58, Canberra, Australia, 1973. Edited by J. R. Shakeshaft. Pp. xv+441. (Reidel: Dordrecht, Holland; Boston, Massachusetts, 1974.) Cloth Dfl. 135, \$54.00; paper Dfl. 100, \$38.50.

THE publications which arise from IAU symposia are among the most valuable books in any library used by astronomical researchers, and the present round is appearing with commendable speed, given the usual timescale for publication of proceedings volumes. Little has happened since August 1973 to reduce the value of these contributions from the leading lights in cosmology and cosmogeny who were then assembled in Canberra; on the other hand, by the very nature of the book many of the contributions have a familiar ring about them.

The editor has rearranged the record of discussion to keep comments near the papers to which they apply, and has also changed the order of presentation of the contributions to fit a more logical pattern than the exigencies of the Canberra timetable allowed. But he has not attempted to provide an overview of the state of the art, and in the field of galaxy formation more than any other in astronomy (except, perhaps, cosmology) a chapter providing such guidance would be invaluable to readers not already thoroughly

familiar with the state of play.

It is no longer very unlikely that non-specialists will take an interest in the areas of study covered by this book, because the high energy astrophysical aspects, in particular, are receiving increasing attention from theoretical physicists who have a background in non-astronomical specialities. But even the cognoscenti have a real problem in distinguishing the wood of a broadly based understanding of how the matter in the Universe got into its present overall state from the many trees of particular theories which explain observations of particular objects or groups of objects without being fitted satisfactorily to the overall pattern.

If you are one of those theorists beaver away on some aspect of the overall problem, then you will find this volume useful not only in providing other models for comparison but in giving some of the observational constraints which must be met by all models. In addition, most active modellers will gain some comfort from the present state of the art; regrettable though it may be to those who treasure the idea of the Universe as a basically simple place, any overview of the work described here must surely lead to the conclusion that both the extreme cases—expansion or outbursts from nuclei, and collapse or condensation of gas clouds—play a part in the real Universe. And there are the various cases of interactions between galaxies to confuse the picture. **John Gribbin**

Organelle by organelle

The Cell in Medical Science. Vol. 1: *The Cell and Its Organelles*. Pp. xiii+366. £7.80; \$20.25. Vol. 2: *Cellular Genetics, Development and Cellular Specialization*. Pp. xvii+597. £11.30; \$29.25. Edited by F. Beck and J. B. Lloyd. (Academic: London and New York, January 1975.)

THESE are the first two volumes of a four volume series on *The Cell in Medical Science*, which is primarily intended to provide the basis of a cell-science course for advanced biological sciences students and for medical students specialising in cell biology during an elective period or during an intercalated year leading to an honours degree.

Volume 1 is concerned with the generalised cell unit and with the biophysical and biochemical basis of the structure and function of the chief subcellular organelles. Its eight chapters deal with the cell as a unit, membranes and transmembrane transport, the cell surface, the nucleus, cytomembranes and ribosomes, mitochondria and peroxisomes, and subcellular pathology. Though rather poorly illustrated, this volume contains a great deal of useful information and its chapters are mostly well written. That by the editors is a particularly well-balanced treatment of lysosome structure and function and lysosome pathology, pharmacology and toxicology. In my opinion, however, this volume fails to indicate (in spite of promises in the Preface) "the directions in which contemporary cell biology is moving and the methods it uses". This is because, with its traditional, anatomical, organelle-by-organelle approach, little attention is given to the current topics which excite cell biologists—for example, cell fusion and cell hybridisation, cell cooperation, nuclear

transplantation, the translation of mammalian mRNA in amphibian oocytes, and the use of cell and organ culture techniques.

The first half of Volume 2 is concerned with cell genetics and development, and begins with four excellent, informative and readable chapters on biochemical genetics, general mammalian cytogenetics, human cytogenetics, and sex chromatin. A very useful chapter on the kinetics of cell production is followed by considerations of cell differentiation, growth and morphogenesis, and ageing. The remainder of Volume 2 comprises comprehensive and, on the whole, well-illustrated chapters on the main cell types and special structures of the neuromuscular system, including the neurone, the synapse, satellite cells of neurones, the skeletal muscle cell, the muscle spindle, and the smooth muscle cell. This theme of morphological adaptation for specific functional purposes will also occupy the whole of Volume 3, in which connective tissues, various endocrine cells, and absorptive and secretory cells will be considered.

In many of the chapters in these first two volumes, medical students would have appreciated more information on cellular aspects of abnormal and disease conditions, at the expense of some of the biochemical, biophysical, physiological and anatomical detail. The balance will to some extent be restored by the publication of Volume 4, which, following chapters on metabolic control mechanisms, will deal with the relationship between the cell and its environment in certain pathological states, including immunological processes, inflammation, wound healing, and carcinogenesis.

The Cell in Medical Science is an essential addition to all medical school and science libraries, where it will be valuable for teachers and research workers, as well as for senior undergraduates.

Michael Balls

Politics at sea

New Era of Ocean Politics. (Studies in International Affairs, No. 22.) By Ann L. Hollick and Robert E. Osgood. Pp.xi+131. (Johns Hopkins University Press: Baltimore and London, April 1975.) £4.40 cloth; £1.40 paper.

THESE studies are confined to US policies and interests in the oceans, the first having particular reference to the Third United Nations Conference on the Law of the Sea and the effect that the different proposals developed there will have for the US. Apart from a little more than one page entitled "International Perspective", however, no attempt has been made to look at the policies from a global standpoint. Having said this, however, it should be remarked that the studies are a very useful contribution to the literature on the Law of the Sea and every participant in the UN Conference should own a copy, or at least have access to one, so as to gain an insight into both the US position in their particular fields and the reasoning behind US statements and activities.

Ann Hollick's study is of greater interest to the marine scientist even though, as is inevitable in a field such as this, it was partially obsolete before it was published, dating from the end of the third (Caracas) session of the Conference in mid-1974; even the outcome of that session is only lightly touched upon. Much, too, has since happened at the recent, fourth session in Geneva.

Consideration of the US position on Marine Pollution and Scientific Research has been confined to some six pages which discuss, first, the reason for including marine pollution within the competence of the conference (this happened because of Canadian insistence after the Manhattan voyage through the North-West Passage in the summer of 1969) and second, how late in the proceedings (summer 1973) the US formulated a policy of Scientific Research; before that, US policy had been almost entirely dominated by petroleum-defence interests. Nonetheless, the US was one of the first countries seriously to consider marine scientific interests at all.

The second study, by Robert Osgood, is an interesting account of US security (that is, military) interests in the oceans, and the surrounding legal implications. This section is of lesser concern to the marine scientist though it does show why US policy has developed as it has, sometimes to the detriment of scientific interests.

The book is well produced and readable in spite of its specialist interest. This is partly a result of a number of fascinating insights into the workings of US government (no doubt others operate in similar fashion but one seldom gets the opportunity to read about them).

Desmond P. D. Scott

Elements of pollution

Chemistry and Pollution. Edited by F. R. Benn and C. A. McAuliffe. Pp. 156. (Macmillan: London and Basingstoke, July 1975.) £8.95.

OVER the past decade there has been much talk of pollution in all its variations, unfortunately in many instances from individuals who were unaware of even the most basic knowledge of the processes involved. This book provides an introduction to the chemistry of the processes of pollution aimed at those individuals with a background of GCE "A" level chemistry; first-year university chemistry in North America. To

obtain adequate in-depth coverage the editors have called on eight contributors to write the six chapters.

The stated objective of the text is to provide a factual chemical background to pollution. As might be expected, with eight authors the depth of coverage in each chapter is rather uneven and this is perhaps the most irritating aspect of the book. Reference to literature and events outside the UK is rather limited and this might limit its acceptance abroad. The authors recommend the book to universities and colleges for use in environmental pollution courses at the lower undergraduate level. It is adequate for this task. There are no problems set. The book is well produced and has an adequate index.

M. A. Ward

obituary

Gordon Hamilton Fairley, a leading authority in the drug treatment of cancer, was killed by a bomb explosion in London on October 23. He was 45.

PROFESSOR Hamilton Fairley began his clinical studies at St Bartholomew's Hospital, London, qualifying in 1954. From 1958–61 he held the Leverhulme Research Scholarship of the Royal College of Physicians. During that time he began his research into the immune mechanisms of blood diseases. In 1965 he was appointed to the consultant staff at St Bartholomew's, where in 1970 he became director of the Imperial Cancer Research Fund Medical Oncology Unit. He was appointed professor at the unit two years later. Much of his time was spent in work on tumour immunology and in improving drug combinations used in the treatment of leukaemias and related disorders, such as Hodgkin's disease.

As reality replaces grief, the magnitude of the loss of Gordon Hamilton Fairley becomes increasingly clear, and facets of his work, taken for granted, suddenly become irreplaceable. It seems difficult to realise that only a decade has elapsed since Gordon directed his attentions to a new approach to cancer research. Following his sound clinical training in oncology, gained under Sir Ronald Bodley Scott, he chose to develop a close liaison with pure scientists to develop a rationale for future clinical studies. During this period he

became a close friend of Peter Alexander, a scientist without clinical training, and between them they taught each other much of their respective disciplines. Basic scientific observations were rapidly reported, particularly in tumour immunology, and tested in clinical programmes. One of his major contributions was to lay down rigid principles concerning these clinical programmes, particularly the concentration of efforts into only a few diseases at any one time, and the use of carefully controlled randomised clinical trials. He had the remarkable knack of keeping the programmes relevant, while keeping an open mind for scientific developments that were important. Using these principles his contribution to the fields of leukaemias and lymphomas was considerable. He had the ability to be effective without making enemies, because of a personality and charm with a common purpose, without interjective smile could shorten committee meetings by hours. It meant that a unit was created which was bound together with a common purpose, without internal squabbles; and his generosity to those who worked for him instilled into them a quality they passed on to others. He was responsible for training half the medical oncologists at present practising in Britain, and his monument will be the living contribution yet to be made by these people. All these qualities made Gordon an obvious choice to steer the direction in which medical oncology takes in Britain. Because he was so young, in this he is irreplaceable. With his death we lose

the founder of British medical oncology, but more tragically to many of us we lose a dear friend.

Ray Powles

B. A. Southgate, CBE, a leading British researcher in problems of water pollution, has died at the age of 71.

Shortly after leaving Cambridge, he joined the recently formed Water Pollution Research Organisation (part of the former Department of Scientific and Industrial Research) as a member—and ultimately leader—of a team investigating the effects of pollution on the Tees estuary. Subsequently, many other such surveys were carried out under his direction, the most important of which was that concerned with the Thames estuary. A predictive mathematical model was developed to establish the best way of improving conditions in the estuary. This research was to form the basis of a new kind of approach to river management. At the outbreak of World War II, Dr Southgate set up the first permanent Water Pollution Research Laboratory (WPRL) in the UK at Watford and was appointed director in 1943. In 1948 he published *Treatment and Disposal of Industrial Wastewaters*, for many years one of the standard reference works. He was awarded a CBE in 1954, the year in which his laboratory moved to Stevenage. After retiring from WPRL in 1966 he continued to act as a consultant to relevant organisations in the UK and in the United Nations.

announcements

Appointments

D. A. Hamburg has been appointed president of the Institute of Medicine, Washington, D.C.

The new pro-vice-chancellor of the City University, London is to be **J. C. Levy**.

E. C. Melby, Jr., has been appointed chairman of the Institute of Laboratory Animal Resources of the National Research Council in Washington, D.C.

Award

The Gairdner Foundation of Ontario has presented one of its 1975 awards to **H. E. Huxley** in recognition of his outstanding contribution to our understanding of the molecular basis of muscle contraction.

Miscellaneous

A workshop on **biological surveys of estuaries** is to be held at UWIST, Cardiff, UK from January 5–9, 1976. A limited number of workshop places are available for river biologists. For further details apply to:—Technical

Presentation Manager, Water Research Centre, Stevenage Laboratory, Elder Way, Stevenage, Herts, SG1 1TH, UK.

Scientific visits to the Soviet Union, Czechoslovakia, Hungary, Romania and Bulgaria. Cultural agreements with the above countries provide for short visits (two weeks) and medium term research visits (two to six months) for British scientists. Scholarships are also available for periods of up to one year. For full details apply to:—Higher Education and Science Department, The British Council, 10 Spring Gardens, London SW1A 2BN.

Reports and publications

Other countries

Annals of the South African Museum. Vol. 67, Part 5: The Amphipoda of Southern Africa. Part 5: The Gammaridea and Caprellidea of the Cape Province West of Cape Agulhas. By C. L. Griffiths. Pp. 91-181. R.5.60. Vol. 69, Part 2: Thermally Anomalous Late Pleistocene Molluscs from the South-Western Cape Province, South Africa. By Anthony J. Tankard. Pp. 17-45. R.2.90. (Cape Town: South Africa Museum, 1975.) [199]

Deutscher Wetterdienst. Deutsches Meteorologisches Jahrbuch. Bundesrepublik, 1973. Pp. xxxvi+240. (Offenbach a.M.: Deutscher Wetterdienst, 1975.) [199]

United States Department of the Interior: Geological Survey. Professional Paper 855: Geology of the Sage Abd Kemmerer 15-Minute Quadrangles, Lincoln County, Wyoming. By William W. Rubey, Steven S. Oriel and Jishua I. Tracey, Jr. Pp. v+18+2 plates. \$2.25. Professional Paper 867A: Stratigraphy of Paleozoic Rocks in the Carlin-Pinon Range Area, Nevada. By J. Fred Smith, Jr., and Keith B. Keiner. Pp. iii+87+2 plates. (Washington, DC: Government Printing Office, 1975.) [228]

The Year Book of the Indian National Science Academy, 1974. Pp. x+179. (New Delhi: Indian National Science Academy, 1975.) [239]

Harvard University: School of Public Health. Dean's Report 1974. Pp. 51. (Cambridge, Mass.: Harvard School of Public Health, 1975.) [239]

World Health Organisation. Technical Report Series No. 572 Education and Treatment in Human Sexuality—The Training of Health Professionals. (Report of a WHO Meeting.) Pp. 33. (Geneva: WHO; London: HMSO, 1975.) Sw. fr. 6. [249]

41st Annual Report of the Director of Sea Fisheries for the calendar year 1973. Pp. 25. (Sea Point, Cape Town: Sea Fisheries Branch, 1975.) [249]

Smithsonian Contributions to Paleobiology. No. 22: Ultrastructural Studies on Graptolites. 2—The Periderm and Its Derivatives in the Graptoloidae. By Adam Urbanek and Kenneth M. Towse. Pp. iii+24+24 plates. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) \$1.30. [259]

Bulletin of the Florida State Museum. Biological Sciences. Vol. 19, No. 3: The Osteology of *Microgobius signatus* Poey (Pisces: Gobiidae), with Comments on Other Gobiid Fishes. By Ray S. Birdsong. Pp. 135-187. Vol. 19, No. 4: The Rock Iguana *Cyclura pinguus*, on Anegada, British Virgin Islands, with Notes on *Cyclura ricordi* and *Cyclura cornuta* on Hispaniola. By W. Michael Carey. Pp. 189-234. Vol. 19, No. 5: Ecological Analysis of the Cayman Island Avifauna. By David W. Johnston. Pp. 235-300. (Gainesville: Florida State Museum, University of Florida, 1975.) [269]

Smithsonian Contributions to the Earth Sciences. No. 14: Mineral Sciences Investigations, 1972-1973. Edited by George S. Switzer. Pp. 88. \$2.50. No. 15: Sands in the Alboran Sea—a Model of Input in a Deep Marine Basin. By Daniel Jean Stanley, Gilbert Kelling, Juan Antonio Vera and Harrison Sheng. Pp. 51. \$1.20. Smithsonian Contributions to Paleobiology. No. 23: Paleornithology of St. Helena Island, South Atlantic Ocean. By Storrs L. Olson. Pp. iv+49 (6 plates). \$1.35. Smithsonian Contributions to Zoology. No. 176: The Biological Investigation of Malpelo Island, Colombia. Edited by Jeffrey B. Graham. Pp. iii+98 \$2.20. No. 196: Calanoid Copepoda of the Family Euchaetidae from the Gulf of Mexico and Western Caribbean Sea. By Taisoo Park. Pp. iii+26. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [269]

Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIV, No. 14: A spectroscopic investigation of the Pleiades. By J. A. Pearce and Graham Hill. Pp. 319-343. (Victoria, BC: Dominion Astrophysical Observatory, 1975.) [299]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. Report No. 13: Waterborne Dissolved Oxygen Requirements and Criteria with Particular Emphasis on the Canadian Environment. By John C. Davis. Pp. xiv+111. (NRCC 14100). (Ottawa: Publications, NRCC/CNRC, 1975.) \$2. [299]

Organisation for Economic Co-operation and Development. Road Research. Roads and the Urban Environment. (Proceedings of the Symposium on Road and the Urban Environment held at the Ministry of Public Works in Madrid on 14th, 15th and 16th October 1974.) Pp. 191. (Paris: OECD; London: HMSO, 1975.) £28. £3.10; \$7. [299]

Smithsonian Contributions to Zoology. No. 199: A Preliminary Analysis of the Intergeneric Relationships of the Frog Family Leptodactylidae. By W. Ronald Heyer. Pp. iii+55. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [299]

World Health Organisation. WHO Offset Publications. No. 18: The Traditional Birth Attendant in Maternal and Child Health and Family Planning—a Guide to Her Training and Utilization. By Maria de Lourdes Verdesse and Lily M. Turnbull. Pp. 110. Sw. fr. 18. No. 20: Guide to the Integration of Health Education in Environmental Health Programmes. By K. A. Pisharoti. Pp. iv+81. Sw. fr. 15. No. 21: Training of Medical Laboratory Technicians—a Handbook for Tutors. By Alex McMinn and Graham J. Russell. Pp. 83. Sw. fr. 15. (Geneva: WHO; London: HMSO, 1975.) [299]

United States Department of the Interior: Geological Survey. Professional Paper 889: Precambrian and Lower Ordovician Rocks in East-Central Idaho. By Edward T. Ruppel. Pp. 34. (Washington, DC: US Government Printing Office, 1975.) [299]

Person to Person

London-Washington. Family house (three young children) sought in Bethesda (NIH)-Washington area for 3 months in exchange for three-bedroom North London house (Dr David Brown, Department of Pharmacology, The School of Pharmacy, London University, 29/39 Brunswick Square, London WC1N 1AX, UK; (01-837 7651 ext. 55).

London-South Carolina. Two- or three-bedroom house wanted in London area for professor on sabbatical from May until December 1976 in exchange for four-bedroom house near South Carolina University. Car may be included (Professor J. L. Safko, Department of Physics, University of South Carolina, Columbia, S.C. 29208, USA).

Eggshell thinning in Falco. Eggshell thinning of several species of raptorial birds has been demonstrated in the British Isles and North America. Drs David Peakall and Lloyd Kiff are attempting to document this change on a global scale. They would be interested to know the whereabouts of any post-1945 eggs of *Falco peregrinus*, *F. biarmicus*, *F. jugger* and *F. cherrug*. The only exception is *F. peregrinus* collected in the British Isles. The measurements required—length, breadth and weight—can be obtained easily without damage to the specimen. They would be grateful if anyone knowing the location of these eggs would write to them (David B. Peakall, Canadian Wildlife Service, Department of the Environment, Ottawa, Ontario K1A 0H3, Canada, or Lloyd F. Kiff, Western Foundation of Vertebrate Zoology, 1100 Glendon Avenue, Los Angeles, California 90024, USA).

Human zygotes. Information is required on a hypothesis on the sex of human zygotes. For this purpose will anyone with data on the sex of any monoamniotic twin pairs please communicate with Dr W. James, Galton Laboratory, University College, London, UK.

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Drug and Alcohol Dependence, Vol. 1, No. 1, September 1975. Edited by H. Halbach. Pp. 1-88. Published bi-monthly. Subscriptions: Vol. 1, SFrs. 115; Personal SFrs. 55. (Lausanne: Elsevier Sequoia, SA, 1975.) [309]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Building Research, 1973/1974. Pp. 133. (East Melbourne: CSIRO, 1975.) [309]

Smithsonian Contributions to Zoology, No. 189: An Annotated List of the Marine Mollusks of Ascension Island, South Atlantic Ocean. By Joseph Rosewater. Pp. iv+41. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office. \$1.25.) [309]

Australia: Commonwealth Scientific and Industrial Research Organisation. Division of Chemical Technology—Research Review, 1973/1974. Pp. 132. (South Melbourne: CSIRO, 1975.) [309]

Great Britain

Awards for Commonwealth University Staff, 1976-78. Pp. 200. (London: The Association of Commonwealth Universities, 1975.) £2.25. [110]

Association of County Councils. Association of Metropolitan Authorities. Association of District Councils. Association of Public Analysts. Joint Survey of Pesticide Residues in Foodstuffs sold in England and Wales, 1st January 1972-31st December 1972 (third year). Report prepared by P. S. Hall and J. H. Hamence. Pp. 36. (Ruislip, Middx.: Association of Public Analysts, 1975.) £4.75. [110]

The Zoological Record. 1971, Vol. 108, Section 6: Vermes, Part A. Compiled by the Staff of the Zoological Society of London. Pp. x+529. £7.50. 1971, Vol. 106, (Section 6: Vermes, Part B. Compiled by the Staff of the Zoological Society of London. Pp. vii+116. £3.75. 1971, Vol. 108, Section 11: Trilobita. Compiled by Sir James Stubblefield. Trilobitoidea. Compiled by Staff of the Zoological Society of London. Pp. vii+67. £6. (London: The Zoological Society of London.) [110]

Countryside Commission—Descriptive Brochure. Pp. 16. (Cheltenham: Countryside Commission, Crescent Place, 1975.) [210]

Forestry Commission. Bulletin 51: Forest Products in the United Kingdom Economy. By B. G. Jackson. Pp. xi+119. £1.35 net. Bulletin 52: Influence of Spacing on Crop Characteristics and Yield. By G. J. Hamilton, and J. M. Christie. Pp. iv+91. 80p net. Bulletin 53: Production and Use of Tubed Seedlings. By Alan J. Low. Pp. vii+46. £1 net. (London: HMSO, 1974 and 1975.) [210]

Republic of Ireland: National Drugs Advisory Board. Annual Report, 1974. Pp. 38. (Dublin: National Drugs Advisory Board, 57C Harcourt Street, 1975.) [610]

Proceedings of the Royal Irish Academy. Vol. 75, Section A, No. 13: The Fredholm Spectrum on Tensor Products. By M. Schechter and M. Snow. Pp. 121-127. 33p. Vol. 75, Section B, No. 21: Observations on the Distribution of the Freshwater Leeches (Hirudinea) of Ireland. By T. K. McCarthy. Pp. 401-451. £1.20. No. 22: Non-Symbiotic Nitrogen-Fixing Bacteria in Irish Soils. By P. M. Murphy. Pp. 453-464. 32p. No. 23: The Biosynthesis of Senecioid Acid. By D. G. O'Donovan and D. J. Long. Pp. 465-468. 15p. No. 24: Recent Changes in the Coastal Geomorphology of the Magilligan Foreland, Co. Londonderry. By R. W. G. Carter. Pp. 469-497. 72p. (Dublin: Royal Irish Academy, 1975.) [610]

National Research Development Corporation. 26th Annual Report and Statement of Accounts, 1974-1975. Pp. 44. (London: NRDC, 66/74 Victoria Street, SW1, 1975.) [710]

University of London. University College Calendar, 1975/1976. Pp. 188. (London: University College London, 1975.) [810]

Proceedings of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 345, No. 1643: A Discussion on the Introduction of Satellites Into Education Systems. Pp. 431-610. (London: The Royal Society, 1975.) UK £3.20; Overseas £3.50. [810]

Department of the Environment: Library Services. DOE Annual List of Publications, 1974. Pp. vi+141. (London: DOE Library Services, 2 Marsham Street, SW1, 1975.) [1010]

British Overseas Trade Board. Report 1975. Pp. 36. (London: British Overseas Trade Board, 1 Victoria Street, SW1, 1975.) [1010]

University of Reading. National Institute for Research in Dairying: Report 1973-74. Pp. 141. (Shinfield, Reading: University of Reading, 1975.) £1.20. [1310]

The Journal of Meteorology, Vol. 1, No. 1, October 1975. Edited by Dr. G. T. Meaden. Pp. 1-44. Subscription, including surface post to UK or any other place in the world (but airmail to the European continent) £6.50 or \$16 US or 70 NF. Single issue rate 60p or \$1.50 US. Subscription, including overseas airmail (countries beyond Europe) £10 or \$24. (Single issue rate 85p or \$2.20 US). (Trowbridge, Wiltshire: Arteteck International, Cockhill House, 1975.) [1510]

Science Research Council. Catalysis: SRC Working Party Report. Pp. 23. (London: Science Research Council, 1975.) *gratis*. [1610]

Office of Health Economics. Medicines Which Affect the Mind. Pp. 44. (London: Office of Health Economics, 1975.) 25p. [1610]

British Gas Research and Development Booklet. Pp. 20. (London: Publications Department British Gas, 59 Bryanston Square, W1, 1975.) *gratis*. [1610]

Royal Observatory Bulletin. No. 179: Provisional Positions of the Moon 1958-1971. By K. C. Blackwell, J. V. Carey and R. H. D. Swift. Pp. 305-320. (Hertsmouneux: Royal Greenwich Observatory, 1974.) 45p net. [1710]